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INVESTIGATIONS OF THE HARDENING PROCESS IN VEGETABLE PLANTS

BY

JOSEPH TOOKER ROSA, JR., B. S., M. S.

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J. T. Rosa, Jr.

This study was undertaken as one phase of a project on the transplanting of vegetable plants. The hardening process, whereby vegetable plants are made more resistant to cold and better able to withstand the hardships of transplanting from greenhouse or hotbed to the open field, is of great importance in the practice of growing certain vegetables which are customarily transplanted. In the production of early crops, hardiness also is especially important because of the low temperatures to which transplanted plants are exposed upon their removal to the field in early spring.

Furthermore, since the hardening process in vegetable plants results in a condition of *acquired hardiness,* developed rather quickly by subjecting plants to certain treatments, experiments with such material throw considerable light on the general problem of cold resistance in plants. This question, in connection with that of the nature of the process of killing of plants by low temperature, has received the attention of numerous investigators during the past one hundred years. Though much information has been accumulated, the whole problem is in a somewhat undefined state. It is the purpose of this paper to propose a theory comprehensive enough to explain satisfactorily the known facts as to the cold-resistance of living plants and to present data on the nature of the response of plant-tissues to treatments which result in increased hardiness. The injurious effects of temperature slightly above the freezing point on the growth of plants are not dealt with in this paper.

REVIEW OF LITERATURE.

The Physical Process of Freezing in Plants.—An early theory as to killing of plants by cold, advanced by Duhamel and Buffon[27*] in 1737, held that death was due to the rupture of the tissues, bursting of the plant cells, by the expansion of ice crystals forming within the cells upon freezing.

*This and subsequent superscript numerals refer to literature cited in the Bibliography.

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Geoppert[82] in 1829, found that ice formation upon freezing of plant tissue was not confined to the interior of the cells and concluded that the killing of plants by cold was not due to cell rupture. A few years later, Morren[73] substantiated Geoppert's conclusion in that he found no organ of the plant torn by freezing. He considered that injury from freezing was due mostly to the separation of air from the plant sap. In 1860, Sachs[105] using improved technique, observed that in the process of freezing, water was withdrawn from the cell and ice-crystals formed for the most part in intercellular spaces.

In 1860, Nageli[89] showed by calculation, that the expansion caused by freezing all the water in the cell, would not be sufficient to cause a rupture of the cell-wall. Prillieux[99] in 1869, found that water was extruded from the cells upon freezing. Müller-Thurgau[74] found that ice formed within the cell to some extent, when the lowering of the temperature was very rapid, but in case of gradual cooling to the point of ice formation as in nature, the crystals were found exclusively in the intercellular spaces. Wiegand[28] noted similar results upon freezing *Spirogyra* and *Nitella.* Thus the finding of ice crystals within the cells by earlier investigators, who froze the plant tissue very quickly, is explained. Cavallero[19] confirmed the work of the German writers, as he found that cell rupture in winter was very rare, the cells themselves never freezing, though ice formation occurred in the intercellular spaces of both hardy and tender plants.

Geoppert[82] noted that plants which were frozen to death lost water rapidly upon thawing. Sachs[106] observed that upon thawing, water remained in the intercellular spaces until reabsorbed by the cells or lost by evaporation. Under certain conditions considerable time elapsed before the water was reabsorbed and the protoplast regained its turgid condition. Prillieux[100] describes experiments on freezing pieces of potato and beet, showing that water was lost from these tissues upon thawing. He recognized also that water was lost from the tissues while still frozen, by evaporation from the surface of the ice crystals.

Prunet[101] found that moisture is lost by evaporation from the surface of the leaf on thawing, rather than by normal transpiration through the stomata.

Abbe[1] stated that as plant tissues were cooled, water exuded from the cells into the intercellular spaces, and after sufficient under-

cooling, this water froze. The concentrated sap left within the cell did not freeze until cooled still lower.

If the water is withdrawn from the cell before freezing in the intercellular spaces, it is important to find how this withdrawal takes place. Wiegand[131] offered two theories to account for cellular water loss upon freezing, "extrusion" and "attraction."

Extrusion.—This hypothesis is that the cell actively gives up water at low temperature by contraction and squeezing. Greeley[84] showed that cooling to near 0°C. caused *Stentor* to contract and become cyst-like. Under the same conditions *Spirogyra* became much plasmolyzed. Livingston showed that when mounted in oil, this plasmolysis was accompanied by extrusion of droplets of water. Wiegand thought that the most probable explanation of this method of water loss from the cell was by change in permeability of the protoplast to the sap solute. A recent report by Pantanelli[96] supports this idea. In experiments with the pericarp of the mandarin cooled almost to the freezing point of this material (–6°C.) he observed a progressive increase in cellular permeability, as shown by rapid loss of water and exomosis of substances from the tissue. Osterhout[93] has shown that freezing as well as treatment by various anesthetics, greatly increases cellular permeability.

Attraction.—Wiegand[139] considered his so-called attraction theory as the more probable explanation of water withdrawal from the cell. Thus in ordinary plant tissue Wiegand pictured the following arrangement:

(1) A film of pure, or nearly pure, water adhering to the outer surface of the cell wall, bordering on the intercellular spaces.

(2) The inert cell-wall cellulose material filled with water of imbibition, which is continuous with that of the protoplast.

(3) A more or less narrow strip of protoplasm adhering closely to the inner surface of the cell wall and containing water of imbibition, continuous with that of the vacuole.

(4) The vacuole, containing an aqueous solution of salts, sugars and other substances.

Normally this system is in equilibrium. According to Wiegand, upon lowering the temperature below the freezing point, the film of pure water on the outer surface of the cell walls freezes first. The tendency will then be to restore equilibrium by drawing water from the interior of the cell to replace the surface film. This water will be drawn first from the cell wall, which in turn will draw on the

protoplast, which in turn will draw on the sap in the vacuole. The water of the vacuole is held by the force of solution alone, whereas the cell wall and protoplasm hold water by the stronger force of imbibition. If the temperature remains constant, this readjustment will continue until the force of crystallization is equalled by the increased force with which the remaining water is held within the cell. After equilibrium is established between the forces of crystallization and the water-retaining power of the cell, at any given temperature, no more water freezes unless the temperature is lowered further, thereby increasing the force of crystallization. However, since the force with which the remaining water is held increases rapidly with the progressive loss of water, Wiegand predicted that the amount of water frozen at each successive degree for which the temperature is lowered would be smaller and smaller. This was shown to be approximately true by the experiments of Müller-Thurgau[74] with apples, and the work of McCool and Millar[80] with green plants suggests the same conclusion. Bouyoucos[11] working with soils, found that little more water was frozen at –78°C. than at –6°C.

The foregoing hypothesis as to the conditions under which ice is formed in living plant tissue has been substantiated by work of

EFFECT OF GLUCOSE SOLUTIONS ON COLD RESISTANCE IN SECTIONS OF RED CABBAGE LEAVES.

Temperature	Concentration of Solution.						
	2M	M	M/2	M/4	M/8	M/16	Water
- 5.2°C.	all	living					½ cells alive
- 7.8°C.	all	living			¼ cells alive	single cells alive	all dead
-11.1°C.	all	living		½ cells alive	single cells alive	all dead	
-17.3°C.	all	living	½ cells alive	single cells alive	all dead		
-22.0°C.	all	living	single cells alive	all dead			
-32°C.	½ cells alive	single cells alive	all dead				

Maximow.[66] In extensive experiments with red cabbage and *Tradescantia discolor* he found a marked "protective" action when sections were frozen in solutions of salts, sugars, and other organic materials, provided the substance used was not toxic and its eutectic point did not lie too near the freezing point. Although the conditions of Maximow's experiments cannot be duplicated in nature, his results are of interest. The following table, taken from Maximow's work, is typical of the results he secured.

Evidently red cabbage cells, which ordinarily are killed at a little below –5°C., survive a temperature as low as –32°C. in 2–mol. glucose solution. Maximow concluded that this apparent protective action of the solution could not be explained by the depression of the freezing point, since the resistance to cold always increased with the strength of the solution much more rapidly than this depression. The degree of protection was found however, to be closely related to the eutectic point of the solution, substances having a high eutectic point showing no protective effect. Isotonic solutions of different substances with low eutectic points possessed nearly the same degree of protective action. Maximow found no relation between the rate of penetration of the protective substance and the degree of protection afforded, and that just as much protective action was exerted by the various solutions when sections were immersed in them and frozen immediately, as when the tissue had been soaked several hours in the solution before freezing. (Hence there could have been no effect on cell sap concentration or in preventing precipitation of the cell proteins.)

If we consider Maximow's work in connection with Wiegand's hypothesis of freezing, we have a condition differing from the usual, in that the film of pure water on the outer surface of the cell wall is replaced by a more or less concentrated solution. In the first place, this would lower the initial freezing point somewhat. More important still, the fact that the cell is surrounded by a more or less concentrated solution should mean that in the process of water withdrawal and ice formation at any given temperature, a state of equilibrium between the ice-crystal and the cell system would be reached sooner than in the case of cells not surrounded by such solutions, if the "attraction" theory of water loss as advanced by Wiegand be accepted. Somewhat less water would be frozen at a given temperature in the cells of tissue immersed in salt or sugar solution. If the amount of water frozen per degree of temperature lowering becomes smaller and smaller, it would be necessary for a "protective" solution to

effect a very small reduction in the amount of water freezing at the lower temperatures to enable the cell to stand cooling several degrees below the usual death-point. Recent work by Vass (122) on bacteria leads to the same conclusion. He found a distinct protective action exerted by glycerine and glucose solutions on freezing bacteria, as shown in the following table.

VASS' RESULTS ON FREEZING OF BACTERIA AT -5°C.

Strength of solution	Percent of bacteria killed	
	In glycerine	In glucose
0.00 (water)	96	—
0.01%	92	98
0.05%	87	95
0.1	41	89
0.5	45	74
1.0	0	58
5.0	0	35
10.0	0	4

Vass concluded, in agreement with Maximow, that the protective action of these solutions was due to their power to keep a film of unfrozen water in contact with the outer layer of the protoplast, the plasma membrane.

Nature of the killing of plant-tissue by cold.—From the foregoing review, the evidence appears conclusive that cell rupture cannot be the cause of killing of plants by cold, but that water-loss from the cells by ice formation in the intercellular spaces is an invariable accompaniment in such killing. According to Müller-Thurgau,[76] Molisch[71] and others, death cannot be due directly to absolute cold, and there is little if any evidence of death due to shock or other reaction attributable to "cold-rigor." Thus, both Müller-Thurgau[76] and Voightlander[123] showed that plant tissues could be undercooled several degrees below the freezing point without injury as long as ice formation did not take place. Wright and Taylor[122] have recently shown that potatoes can be cooled several degrees below their freezing point and warmed up again without injury, provided no ice formation took place. However, jarring undercooled potatoes caused ice-formation to take place and resulted in typical frost injury.

Chandler[20] found evidence that tender plants exposed to temperature slightly below freezing when the surface of the leaves was wet, killed to a greater extent than if the leaves were dry. This result is explained by Harvey's "injection" theory, according to which undercooled tissues are caused to freeze in spots where droplets of free water on the surface crystallize and inoculate the tissue just beneath with the growing crystals.

These facts strengthen the view that killing by cold depends on ice formation, rather than on the effect of low temperature in itself. Just how death is caused by the freezing process is a question of interest. Four distinct theories have been advanced.

(*a*) *Direct result of water loss—"desiccation."*—Müller-Thurgau[74] believed that death was the direct result of the water loss, that is, death ensues when so many molecules of water are withdrawn from the protoplast that its living structure is permanently destroyed. Wiegand[131] concurred in this hypothesis, with the additional suggestion that "probably every cell has its critical point, beyond which water withdrawal causes death." Cavallero[19] attributed killing to the wilting upon thawing, due to rapid evaporation of melting ice in the intercellular spaces. He mentioned an opinion generally held by practical gardeners, that under conditions favoring slow thawing or slow evaporation, such as shade or moisture, severe injury to the plant might be prevented by the re-entry of water into the cells. However, Müller-Thurgau[74] and later Molisch[71] found no difference in extent of killing, between rapid and slow thawing. Chandler[20] also concluded from a considerable number of experiments that the rate of thawing generally had no influence on death from freezing. We should distinguish here between the loss of water from the cell and its loss from the plant as a whole. If the cells are killed directly by loss of water on freezing, or if they are killed by changes taking place as a result of this water loss, then the rate of thawing would have no effect on the killing. However plants capable of standing some ice formation within their tissues, would take back more of this water if thawed slowly, whereas they might lose the most of it if thawed rapidly. This explains two things, the wilted condition often observed in frozen plants upon thawing, and the cumulative effect of successive freezing and thawing, whereby a fraction of the plant's water content is permanently lost by the plant on each thawing.

Nelson[90] thought that rapid loss of moisture was the principal cause of winter-killing of shrubs in high, dry sections. Kylin[56] in a recent study of the cold resistance of marine algae, concluded that death from cold was conditional upon actual formation of ice and that such death was primarily due to withdrawal of water from the cell. Matruchot and Molliard[64] described the successive changes in arrangement of the chromatin strands of the nuclei in leaf cells of the snowdrop subjected to freezing temperatures. They stated that water was withdrawn from the protoplast and nuclear material of the cell, and that this continued if the temperature was sufficiently low,

until these portions of the cells contained less water than the minimum necessary to vitality. They[63] also subjected plant tissue to freezing, to drying and to the action of solutions of high osmotic concentrations. They observed a marked parallelism in the effects of these treatments, hence they concurred with Molisch and Müller-Thurgau in that death of the cell was due to rapid loss of water. Adams[3] working with moist seeds, observed that in freezing, water was drawn from the cells and solidified in the intercellular spaces and if the freezing did not go too far, upon melting the water was reabsorbed slowly, without injury having been done to the cells.

Wiegand[129] made extensive observations on the freezing of leaves and buds. In buds in winter: "Ice was always found in broad, prismatic crystals arranged perpendicularly to the excreting surface and usually formed a single continuous layer throughout the mesophyl of the scale or leaf, to accommodate which the cells were often separated a considerable distance. The cells near the ice mass having lost their water, were in a state of collapse, but upon thawing they reabsorbed the water and resumed their normal condition." This was also true of evergreen leaves, in which he observed that ice crystals first lined the spaces of the spongy parenchyma, later filling these spaces and, in leaves of high water-content, the crystals fused into a sheet of ice completely separating the upper and lower portions.

In observations on thawing of frozen leaf sections, Wiegand noticed in hardy tissues, not killed by the freezing, that upon thawing the water was drawn back into the cells, but in tender tissues killed by the freezing process, water was not drawn back into the cells to any extent. Pantanelli[94] concluded that the suffrance of each cell is directly proportional to the outgo of water during cooling. He also attaches great importance to the condition of the roots with reference to ready water absorption in determining whether or not plant recovers from freezing.

(*b*) *Injury to the plasma membrane by water withdrawal.*—Maximow[66] concluded as a result of an extensive series of experiments, wherein sections of plants were frozen in solutions of various salts and inorganic materials, that killing by cold is not due to low temperature as such, but to physico-chemical changes set up in the colloids of the plasma membrane during ice formation therein. This is really a modification of Müller-Thurgau's theory, limiting the injurious effects of water-loss to the outer layers of the protoplast.

Chandler[20] also concluded from his exhaustive researches that

"killing from cold is more likely a mechanical injury due to withdrawal of water from the protoplasmic membrane than an injury resulting from a precipitation of proteins."

(*c*) *Protein precipitation through "salting out."*—Gorke[35] concluded that killing was due to irreversible precipitation of the proteins of the cell. He accounts for this precipitation by the greater concentration of the salts in the sap as water is withdrawn from the cell by formation of ice, since certain proteins are precipitated in strong salt solutions. He found that approximately $1/3$ of the proteins were precipitated in frozen cereal plants. Gorke found also that hardiness of certain plants bears some relation to the ease with which their proteins were precipitated. In the tender begonia he obtained protein precipitation at –3°C., in winter rye at –15°C. and in pine needles at –40°C. Schaffnit[110] also concluded that protein precipitation was the cause of death. He found that the proteins of rye plants grown in the open at low temperature were not as easily precipitated upon freezing as those of tender greenhouse plants. The effect of low temperatures on the hardiness of plants grown in the open was ascribed to a transition from less stable to more stable forms of the proteins by splitting. He found that he could prevent the precipitation of proteins from the sap of tender greenhouse plants by addition of sugar, to which he ascribed a protective action against protein precipitation and consequently against injury of the plant from cold, although it was not proven that these two are always related.

Chandler[20] was rather disinclined to accept the idea of killing by "salting out" of proteins. He found that the hardiness of plants was increased by growing them in salt solutions, such as zinc sulphate, which is an excellent protein-coagulating agent. However, Chandler's work on this point cannot be held to disprove the protein-precipitation idea, since he showed no evidence that the protein-precipitating salts were taken up by the plant, or if they were taken up, that they existed in the plant in a form which would precipitate proteins upon concentration. However, the fact that Chandler did not find appreciable protein precipitation on freezing the extracted sap of apple twigs indicates that killing may not always be accompanied by protein precipitation, although his technique on this point may be open to question.

(*d*) *Protein precipitation by increase in acidity.*—Changes in color of plant sap due to change in reaction upon freezing are well

known. Gorke[35] noted an increase of acidity in sap upon freezing. He believed this was a factor in the precipitation of the plant proteins, since the acidity of the medium is important in determining the state of such colloidal materials.

Harvey,[42] in a recent paper dealing with cold injury to cabbage plants, extended this theory. He found definite evidence of increased acidity as a result of freezing cabbage plant juice, by measuring the hydrogen-ion concentration before, during and after freezing to definite temperatures. He noted protein precipitation when the actual acidity was increased from PH 5.65 to PH 5.26. It is especially interesting to note that Harvey found a similar increase in the acidity of juice expressed from leaves exposed to wilting, though he does not state if the leaves were wilted beyond recovery. Harvey demonstrated that if phosphoric acid was added to the expressed sap until the Hydrogen-ion concentration was increased as much as it would have been by freezing, a precipitation of the protein occurred, thus implying that the parallel effect of water loss by wilting or by freezing and addition of acid, was protein precipitation and death.

Harvey repeated Gorke's experiment on the precipitation of protein from expressed sap by freezing. Samples of juice were taken from hardened and not hardened cabbage plants and frozen to -4°C., a temperature which would kill the non-hardened, but not the hardened plants. It was found that 9.4 percent of the protein in the juice of the hardened plants was precipitated and 31.2 percent in the tender plants. Repeating the experiment and adding sufficient acid to change the reaction of the juice the same amount as it would be changed by freezing to -3°C. he found that 11 percent of the protein was precipitated in the juice of hardened, and 44 percent in tender plants. He also made complete analyses of hardened and tender cabbage plants, finding that of the water-soluble fraction of nitrogen about 35 percent was amino-nitrogen in hardened plants, and only 17 percent in tender plants, having about the same amount of water-soluble nitrogen. Harvey thought this increase in amino-nitrogen to be a very significant result of the hardening process, though he said it was not necessary that complete cheavage of the proteins to the amino acids should occur, to prevent their precipitation on freezing.

Relation of water-withdrawal from the cells to killing by cold.—No matter which agency is chiefly operative in the actual freezing and killing process, they all depend on the withdrawal of

water from the cell. Irreversible coagulation of colloids, such as protoplasm, is itself essentially a dehydration process. It is, then, by means of factors affecting water-withdrawal from the cell by ice-formation that the differential killing of plant tissues by low temperatures may be explained.

Schaffnit[110] classified plants in three groups, according to their cold-resistance and ability to withstand desiccation.

1. *Plants for which water is absolutely essential.* This we take to include such plants as tomatoes, which are killed once extensive ice formation actually takes place.

2. *Plants which withstand a certain degree of desiccation.* These would be such plants as the cabbage which can survive a certain amount of ice-formation in the tissues without injury. It is this group with which we are mostly concerned in discussions of hardening or cold-resistance.

3. *Those which withstand complete drying—seeds, spores, etc.*

This classification can be taken to include all plants, except those which are killed by cold above the freezing point. Such killing is probably due to inability to carry on their normal metabolic functions at low temperatures, as suggested by Molisch, rather than to direct effect of cold.

Relationship to cold resistance of factors influencing the water-retaining power of cells.—If the killing of plant tissue by cold is primarily due to water-withdrawal from the cells beyond a certain minimum point, then the difference between hardy and tender tissues may be ascribed largely to the relative water-retaining power of the cells in the two types of tissue.

There are two main forces concerned in the water-retaining power of plant cells. (1) Osmotic concentration, due to sap solutes in the vacuole, and (2) Imbibition, a force exerted by some constituents of the cell wall, nucleus, plastids, and especially by the colloidal cytoplasm. The importance of either of these forces in the water-retaining power of cells may be influenced by various factors.

Osmotic concentration and water-retaining power.—Since the freezing point of a solution is lowered in proportion to its molecular concentration, several workers have sought a correlation between cold resistance and the molecular concentration of the sap as measured by the depression of the freezing point.

Lindley[61] in reviewing the work of Morron and others in 1852,

was probably the first writer to connect the depression of the freezing point of the sap with cold resistance.

Chandler[20] directed much attention to the relation of osmotic concentration to hardiness, although he admitted that the force of imbibition may be the more important factor in the water-retaining power of plant tissue. He found in most cases that the hardier plants had the more concentrated sap. To explain the relation of a slight difference in freezing point depression to a considerable difference in hardiness, Chandler reasoned that, since in a solution containing one gram molecule the freezing point is -1.86°C., and in a M/2 solution, -0.93°C., in the latter solution at a temperature of -0.93°C. all the water would be unfrozen, at -1.86°C. one half would be unfrozen, and at -3.72°C. one-fourth would be unfrozen, and so on. If this held true for the water contained in a plant, the sap of which is equivalent to about one-half gram molecular concentration, we would then expect 75 percent of the water to be frozen at -3.72°C. However, Chandler's conjecture on this point does not apply in all cases since McCool and Millar found in their dilatometer experiments that nearly as much water is frozen at -4°C. in wheat plants having a freezing point depression of 1.107°C. as in corn plants having a depression of only 0.578°C.

Ohlweiler[92] in studying the effect of a late spring frost on vegetation at St. Louis, found that plants which showed the greater osmotic concentration of the sap were generally injured the least, although there were some exceptions. He found, for example, that in twelve species of *Magnolia*, the order of hardiness paralled the order of sap concentration fairly well. Harris and Popenoe[40] found that on the average, the hardier species of avocado had slightly the greater sap concentration. Lewis and Tuttle[59] working on evergreen leaves in Canada, found that in *Picea Canadensis*, the freezing point lowering varied only slightly from October to April, the maximum lowering being in March. In the bark of *Populus* and the leaves of *Linnaea* and *Pyrola*, the maximum depression of the freezing point was also found to be in March, after the coldest weather was over. The freezing point depression was found to parallel the accumulation of sugars during the winter months, the maximum sugar content being found April 2nd., just before spring growth started. They found little correlation between cold resistance and sap concentration, as measured by the depression of the freezing point. Pantanelli[95] likewise, was unable to establish a relation between osmotic concentration of the cell sap and resistance to cold.

Salmon and Fleming[109] found no relationship between sap concentration and winter hardiness in several common cereal crops in Kansas. Thus on November 27th., hardy Kharkov wheat gave a freezing point depression of 1.230°C. and tender Culberson oats 1.199°C. On December 17th., the freezing point depression of the wheat was 0.935°C. and of the oats 1.260°C. They explain these results by the supposition that oats are less able to secure sufficient water from the soil to supply that lost by transpiration, the ground being frozen at the time of the second determination. This resulted in water-depletion in the oat plants, giving a higher cryoscopic value to their sap.

Wiegand[131] thought osmotic concentration of plant sap to be of importance in relation to ice-formation at the inception of freezing only.

Imbibition and water-retaining power.—The term "imbibition" will be used in this paper in the general sense, as applying to the absorption of water by colloidal materials and the holding of water by finely divided solids by means of surface phenomena, such as adsorption, adhesion or molecular capillarity.

De Candolle[75] (quoted by Lindley in 1855) formulated the following laws of temperature in relation to plants:

"1. The power of the plant to resist low temperature is in inverse ratio of the water content.

"2. Hardiness is in direct proportion to the viscidity of the plant's fluids.

"3. Hardiness is in inverse ratio to the rapidity with which the fluids circulate.

"4. Tenderness is greater in proportion to the size of the cells."

Considering that De Candolle had few or no experimental data from which to draw conclusions, and that he wrote many years before the classical researches of Müller-Thurgau, his views on the resistance of plants to low temperature are remarkably near present conceptions.

Wiegand[131] considered that the force of imbibition was to a large extent the cause of the water-retaining power of plant cells. According to Pfeffer[140] this force increases with decreasing moisture content. Although Wiegand made no quantitative measurements, his theories were the result of keen observation and sound reasoning and are of very great importance to an understanding of the differential killing of plants by cold. He pointed out that the water of crystallization in frozen plant tissue was practically pure, sepa-

rating from the other cell constituents upon freezing. The progressive dehydration of the cell by the withdrawal of water to form ice crystals, was thought by Wiegand to increase the combined forces of osmosis and imbibition holding the remaining molecules of water. He advanced the hypothesis that the degree of cold necessary to form ice was proportional to the force which held the water in the tissues, which force (osmosis plus imbibition) was thought to depend largely on the water content. Wiegand believed that in succulent tissues of high water-content, most of the water would be frozen out near the initial freezing point and a smaller portion would be frozen in less succulent tissues.

Wiegand[129] observed that no apparent ice formation took place in the buds of *Quercus, Castanea, Hicorea, Juglans,* and *Fraximus,* at –18°C. The buds of these species were observed to differ from many others in which ice formation took place at a higher temperature by: (1) lower water content, (2) smaller cells, (3) thicker cell walls. He considered that these factors favored the retention of cell moisture by a relatively greater force of imbibition than in buds lacking such characteristics and in which ice forms at a higher temperature. Wiegand also observed that the ice crystals in frozen beets and potatoes were smaller near the periphery than in the center of these organs. The cells of the peripheral regions in these roots being smaller and poorer in water, were thought to have a greater capacity for retaining water against the formation of ice crystals.

Recent work by Parker[97] strengthens Wiegand's hypothesis that decreasing water content increases the force of imbibition. He found that finely divided materials in suspension held a considerable amount of water as capillary surface films, and the force with which this capillary water was held increased rapidly with decreasing moisture content. That moisture content has a marked influence on the force of imbibition is indicated also by the work of Reinke,[139] who found that a pressure of sixteen atmospheres would squeeze water from a frond of *Laminaria* when the moisture content was 73 percent, but when the moisture content was reduced to 48 percent, it required a pressure of 200 atmospheres to extract water.

If decreasing moisture content increases the force with which water is retained by plant cells, a direct connection is indicated between such water-retaining power and cold resistance, for several investigators working with a wide variety of plants have shown that hardiness is usually associated with low moisture content. Thus, Lindley[61] recognized the fact that decreasing the moisture content

tended to increase cold resistance and that the removal of some water in the "ripening process" made the plant's tissues better able to withstand cold. Detmer[26] stated that such parts of plants as are poor in water withstand low temperature best. He found that air-dry seeds of *Triticum* and *Pisum* germinated normally after exposure to temperature of –5° to –10°C., while turgid seeds were killed under the same conditions.

Gorke[35] noted that the more hardy plants had the greater percentage of dry matter and slightly lower sap freezing point. Schaffnit[110] found a gradation in the amount of dry matter in different varieties of wheat in direct proportion to their resistance to low temperature. He concluded that high dry-matter content was correlated with high frost resistance. Rivera[108] found that all cultural conditions which tended to increase the percentage of dry matter in wheat decreased the tendency to lodging and increased hardiness. Hedlund[44] found that under like cultural conditions, those varieties of winter wheat having a higher percentage of dry matter in autumn are generally more winter-hardy than those having a low percentage. He found also that cultural conditions that make for high percentage of dry matter favor winter hardiness. Hedlund attributed the high dry-matter content of hardy plants to their large carbohydrate content.

Shutt[114] found that a correlation existed between percentage of dry matter and hardiness in apple twigs. A set of samples gathered on the Canadian Experiment Farm in midwinter had moisture contents ranging from 45.1 percent in terminal parts of twigs of Yellow Transparent (hardy) to 51.59 percent in the same portion of the Blenheim Pippin (tender). He recommended the use of cultural practices to regulate the moisture content, as indicated by the degree of maturity in the fall. It is now a pretty well recognized fact that the ability of a variety of the apple to survive in Northern sections depends on its maturing thoroughly before winter—in other words, developing a condition of low moisture content and maximum water retaining power. Webber[127] and his co-workers observed after a very severe freeze in the citrus regions of California that trees and portions of trees which were dormant or inactive were much less injured than those actively growing and functioning. Trees which had been rather dry for some time also were more hardy than those recently irrigated while trees suffering badly from drought were injured worst.

Batchelor and Reed[5] found that winter-injury of the distal end of the branches of the Persian walnut in California could be pre-

vented by bringing the trees to early maturity by with-holding water, followed with heavy irrigation during the winter.

Johnson[51] found a marked seasonal increase in water content of peach buds in Maryland, correlated with the increased tenderness of buds in spring. The variety Greensboro had a lower water content than the Elberta, which is a tenderer variety. West and Edlefsen[128] also working on peach buds, pointed out that buds might escape injury from cold by under-cooling below the freezing point without ice formation, when the amount of moisture in the buds was small.

Chandler[20] and more recently Carrick[18] found that apple roots which had been allowed to absorb moisture for several hours were injured by cold a little more than normal roots, whereas partial drying increased their cold resistance.

Beach and Allen[6] found that drying apple twigs before freezing lessened the injury by cold. They also found that the hardier varieties of apples have the lower moisture content during the growing season but after prolonged freezes in winter, these hardy sorts may contain more moisture than tender varieties. In other words, the hardy twigs undergo a smaller water loss during freezing.

Salmon and Fleming[109] performed an interesting experiment with greenhouse-grown cereal plants, which demonstrated that cold resistance may be increased by decreasing the amount of water in the tissues by slight wilting. Wheat plants were dug up, wilted for two or three hours, and exposed to freezing temperatures. Turgid plants killed much worse than slightly wilted plants at a temperature of –2 to –3°C. for 20 to 30 minutes.

Chandler[20] compared the relative extent of killing by cold in turgid and wilted plants. He included in his experiments a large number of tender plants which are incapable of withstanding ice formation and which cannot be expected to show much response in the way of hardiness to any treatment. His experiments were made in summer, hence the killing at temperatures only slightly below freezing. Though Chandler concluded that on the whole, wilting does not increase cold resistance, yet the following table, taken from his data, indicates that under certain conditions, wilting may do so.

In the case of lettuce, it seems that the wilted plants were killed the worst by slight freezing, –2°C. At the lower temperatures, however, the percentage killed increases very rapidly in the turgid plants, and slowly in the wilted plants, so that the killing of turgid leaves considerably exceeds that of the wilted when the temperature of

EFFECT OF WILTING ON KILLING BY COLD, COMPILED FROM CHANDLER, p. 196.

Plant	Condition	Temperature			
		-2°C.	-3°C.	-4°C.	-4.5°C.
Lettuce	turgid wilted	12½% killed 47% killed		66.6% 55.5%	83% 62%
Red Clover	turgid wilted	17% killed 34% killed	100% 66.6%		
Rose Geranium	turgid wilted	97% killed 60%	100% 100%		
Red Cabbage	turgid wilted			65% 44%	

-4.5°C. is reached. The same thing is indicated in the case of red clover. Chandler remarks that brief wilting does not increase the total amount of material in the cell sap which might function in holding water in solution, yet it seems that the hardiness of the plants may be materially affected.

Wiegand[131] states that the greater the water content, the thicker the film of water on the surface of an imbibing substance, such as the plant cell, and the weaker the force by which the outer layers of this film are held, hence more easily withdrawn to form ice. Parker[97] has furnished some experimental data, which substantiates Wiegand's suggestion.

Kiesselbach and Ratcliff[62] in experiments with seed corn, found that death from freezing was directly proportional to the moisture content of the kernel and to the duration of exposure to cold. Seed corn maturing in the natural way was found to become cold-resistant progressively as the moisture content decreased. The following table taken from their data, illustrates the relation between moisture content and killing by cold as measured by the germination of the seed.

Kiesselbach and Ratcliff found that the temperature as which ice formation commences in the corn kernel depends very largely on the moisture content. Immature seed containing 60 to 80 percent moisture, froze just below 32°F., whereas in air-dry seed, containing 18 percent moisture, no ice formation could be detected at -10°F. Usually where ice formation took place in the seeds and they remained in the frozen condition 24 hours, the vitality was weakened or destroyed, but in some cases ice formation within the seed was not followed by

RELATIVE GERMINATION OF SEED CORN OF VARYING MOISTURE CONTENT AFTER EXPOSURE TO LOW TEMPERATURES. (After Kiesselbach & Ratcliff)

Temperature to which exposed Degrees F.	Percent moisture content of grain									
	10 to 15	15 to 20	20 to 25	25 to 30	30 to 35	35 to 40	40 to 45	45 to 50	50 to 55	60 to 65
32—28			100	85	75	71	69	—	33	0
24—20		100	96	77	67	13	12	12	6	0
16—12		100	88	34	12	0	0	0	0	0
8—4	100	98	47	7	0	0	0	0	0	0
0— -5	97	63	0	0	0	0	0	0	0	0

death. They show that air-dry seed are uninjured by low temperature, and that ice-formation does not take place therein.

The observations of Gorke, Schaffnit, Rivera, Hedlund, Shutt, Webber, Wiegand, Beach and Allen, West and Edlefsen, Batchelor and Reed and Johnson, indicate that individual plants, species or varieties having a low moisture content are usually hardier to cold than those having a high moisture content. The work of Chandler, Carrick, Beach and Allen, Salmon and Fleming, and Kiesselbach and Ratcliff indicates that reducing the moisture content of a given plant or part of a plant increases its cold resistance. This, it seems, may be partly accounted for by Wiegand's hypothesis and Parker's recent work, in that the force with which water is held by plant cells increases with decreasing water content. Removal of some water by drying before freezing should increase the force with which the remaining moisture is held. In other words, if plant tissues become more cold resistant upon slight drying out, such increase in hardiness may be ascribed to the increased power of imbibition on the part of the plant's cells.

Relation of factors influencing water loss by the plant as a whole, to hardiness.—The foregoing discussion has shown the relation of some factors to the water-retaining power of plant tissue, as measured by the effects of low temperature. It is indicated that increasing the water-retaining power of the cell, either by increasing the concentration of its sap, or by increasing its power of imbibition, or both, results in greater resistance to low temperature because of the increased force of crystallization necessary to withdraw the required amount of water to cause death or bring about the changes which cause death. If the ability of the individual cell to retain some moisture when exposed to freezing is the significant point of differ-

ence between tender and hardy tissues, then the plant as a whole may show the same difference in water-retaining power and resistance to water loss, but this does not imply necessarily that hardiness and drought resistance go together. Salmon[108] remarks that some hardy grasses thrive best in damp localities. In drought-resistant species, the plant as a whole may be protected against water-loss by morphological differences in structure, such as special water storage tracts, few or small stomata, thick integument, bark, scales, xerophytic characters in general; yet the individual cell may possess little water-retaining power which would prevent the excessive withdrawal of water upon freezing.

While a low transpiration rate due to morphological modifications would undoubtedly be of great assistance to plants in withstanding injury from physiological drought, a low transpiration rate also may be associated with high water retaining power of the cells.

Beach and Allen[6] observed a loss of four to nine percent in weight of apple twigs during a single week in January with a minimum temperature of -15°F. They found that in general the hardiest varieties are most resistant to the loss of water.

Strausbaugh[117] found that coincident with the breaking of the rest period in semi-hardy varieties of the plum in midwinter, the moisture-retaining power of twigs and buds decreased rapidly, while in the hardy variety Assiniboine, which remained dormant until early spring, the water-retaining power remained constant. This is significant, since increased tenderness to cold, especially of the flower buds, follows the break of the winter rest.

Sinz[115] concluded as a result of experiments at the University of Göettingen that those varieties of winter wheat which seemed able to prevent rapid transpiration, were among those most highly resistant to cold.

Weaver and Morgensen[126] in Nebraska found that in winter the water losses of coniferous trees with their needles intact, are relatively no greater than are the losses from deciduous trees after leaf-fall. This indicates great water-retaining capacity in the foliage of conifers, most of which are very hardy.

Some writers have likened hardy to desert plants because of their xerophytic characters, by which water loss is reduced to a minimum. Thus Schimper[112] states that desert plants frequently have a strong resemblance in their structure and habit of growth to those of polar regions, as would be expected if resistance to cold depended on the reduction of water loss to a minimum. What Schimper probably

had in mind was the form of injury due to physiological drought, where above-ground plant tissues are killed by desiccation resulting from their inability to obtain water from a frozen soil or through a frozen stem.

Storber[118] states that "winter leaves" of herbs are quite xerophytic in structure, enabling them to survive the severe conditions to which they are exposed. He points out a fact that seems to have been hitherto overlooked—that the low water content and high osmotic concentration in hardy plants may insure to them more ready absorption of soil water. This would certainly be of great importance to plants in winter, in overcoming physiological drought, as well as increasing the resistance to the direct effects of freezing. Dachnowski[22] observed xerophytic developments in plants exposed to physiological drought conditions in bogs. Modifications were found enabling certain plants to survive in bogs in spite of slow water absorption due to toxicity of bog waters. The following are the chief modifications to which Dachnowski ascribes resistance to rapid water loss in leaves of bog plants.

1. Reduction in size of leaves.
2. Thick-walled epidermis.
3. Cuticle, wax, and hairs.
4. Mucilaginous and resinous bodies in leaves and roots.

Groom[36] stated that the function of mucilages and tannin in buds is to help hold the water in the young shoots. Chandler[20] found that the bud scales of the peach had no influence on the resistance of the embryonic tissue to low temperature, but that they served as protection against drying out by repeated freezing and thawing. Wiegand[130] recognized that loss of water from the plant might take place by evaporation from the ice masses in frozen tissues, and suggested that bark and bud scales serve as protection against such loss. As pointed out earlier in connection with the rate of thawing, protection against such loss of water would be most important in tissues exposed to repeated freezing and thawing, as buds undoubtedly are in winter.

In a number of recent experiments on the raspberry in Nebraska, Emerson* found that by coating the canes with paraffin, winter-injury could be prevented. He observed that untreated canes killed only to the snow-line. Emerson's results indicate that mechanical protection against loss of water by the plant as a whole,

*Emerson, R. A. Cornell University, Ithaca, New York, Personal correspondence with F. C. Bradford.

may prevent the form of winter injury due to local physiological drought, wherein parts of plants exposed to repeated freezing and thawing and consequently to loss of water which cannot be replaced because of frozen stem or frozen or dry soil, are eventually killed by the progressive desiccation of the tops. This type of cold injury is distinct from the direct effects of low temperature, yet some of the factors which increase the water-retaining power of the tissues in the latter case may also be of importance in enabling the plant to withstand the former.

Irmscher[49] attempted to correlate the cold resistance of certain peat mosses with their ability to withstand long drying out. He found that most species could stand a temperature as low as -20°C., but they were all killed at -30°C. He states that "no thoroughgoing parallel was found between cold resistance and ability to survive long slow drying." However, he found that any particular species could be made more resistant to frost by previous drying out. Mosses growing in a dry location were found more hardy than the same species in moister places. Irmscher attributed to a "regenerative cell-complex" the means by which these mosses were enabled to survive both extreme cold and extreme drying. A higher osmotic concentration and greater cold resistance was observed in species of moss growing at low temperature.

STATEMENT OF PROBLEM.

The work of the earlier investigators shows that freezing to death of plant tissue is associated with water-withdrawal from the cells—the actual death process being due to (a) the direct effect of water subtraction on the protoplast, or (b) precipitation of proteins because of the increased acidity, or (c) precipitation of proteins due to increased salt concentration, or perhaps to other processes which have not as yet received attention.

Regardless of the particular theory which may account for the ultimate killing of plant tissue by cold, the consideration that the primary factor is water-withdrawal logically suggests the following questions. In general, would not cold resistance be proportional to the water-retaining capacity of the plant cells? Since the force of imbibition increases with decreasing moisture content and since also cold resistance in plants increases with decreasing moisture content, does not cold resistance depend largely on the imbibitional force with which the cells retain moisture? Do hardy plant cells actually retain more moisture when exposed to freezing than cells of tender

plants? Do tender plants exposed to hardening treatments acquire an increased cell-water-retaining power, and if so, is this the main factor concerned in their increased cold resistance? Also, how is this increased water-retaining power acquired and what changes in the living plant are concerned therein? In order to answer these questions, the following experimental work has been undertaken.

EXPERIMENTAL WORK.

Materials used.—Most of the experiments were performed with the cabbage, as a representative of a type of plant which is capable of being hardened so as to withstand considerable ice formation within the leaves. Leaf lettuce, head lettuce, kale, cauliflower and celery were used to some extent. These also are plants capable of being hardened so that they can be frozen stiff without injury.

The tomato was used as the principal representative of a type of plant which cannot be hardened so as to withstand ice-formation, but which is capable of hardening to the extent that the freezing point is lowered slightly. Other plants used of this type were peppers, eggplant and sweet potatoes.

In each series of experiments plants of the same variety and age were used.

Methods of hardening.—*Series E.*—The plants were kept in a warm greenhouse until nearly large enough for transplanting to the garden. The plants to be hardened were then removed to an open coldframe where they were exposed to temperatures near freezing during the night and to full sunlight during the day. This method of hardening was followed both in early spring and in late fall. Samples were gathered for analysis usually at intervals of 5, 10 and 20 days after the beginning of the hardening treatment, as well as from some of the original lot of plants which had been kept in the greenhouse under favorable growing conditions. The soil moisture supply was kept as nearly as possible the same for the plants in the greenhouse and those being hardened in the frames, so that temperature would be the principal limiting factor in their development.

Series A.—The soil moisture for plants grown in a warm greenhouse was varied. As soon as the seedlings were well established after transplanting from the seed flat, a number of potted plants of uniform size were selected and divided into lots which were given

different treatment only in so far as water supply was concerned. One lot, A1, was given liberal moisture—these plants were kept in rapidly growing condition and were always the tenderest plants in the experiments. Another lot, A2, was given moderate moisture, so that the plants grew at a moderate rate. Another lot, A3, was given just enough water to keep the plants growing slowly. They frequently wilted somewhat in the middle of warm, bright days. This lot usually showed nearly the same degree of hardiness as those plants that had received the maximum degree of hardening in the coldframe. A fourth lot, A4, was included in some of the experiments, these plants being watered liberally at first, then water was partially withheld for a week or ten days before samples were taken.

Series B and C.—Plants were grown under uniform conditions in the greenhouse, in soils of different composition made up by mixing different proportions of sand and compost. Few data are reported on this series because it was found difficult to maintain uniform moisture conditions in soils of such diverse texture. Also other factors, such as degree of root binding, were likely to become limiting before excess or deficiency of nutrients could exert much effect. However, it was definitely shown that growing plants in poor soils would increase their cold resistance, other conditions being the same. Such plants were smaller and grew more slowly than the more tender plants in the better soils. This series of experiments might have been more successful if the plants had been grown in a uniform soil-medium to which varying quantities of nutrient solution were added.

Series H.—The treatment consisted of severely pruning the roots by running a knife close to the stem on one or both sides of the plant. This treatment checked the growth of the plants quite materially for a short time and increased the cold-resistance somewhat.

Series F.—A quite effective method of hardening was watering with M/10 solutions of various salts. The plants were grown under uniform conditions in a warm greenhouse and the test lots were watered with the various salt solutions whenever the soil became rather dry or whenever the plants wilted badly. In some cases, as under high transpiration conditions, the wilting point was reached while the moisture content of the soil was high. It is not altogether clear whether the hardiness resulting from these salt applications was due to their specific action, to a condition of mild physiological

drought, or to the toxicity of such concentrated solutions to the roots. This will be discussed in more detail later.

EFFECT OF HARDENING TREATMENTS ON PLANTS.

External appearance.—*Cabbage.*—Tender (wet-grown) greenhouse plants were usually about twice as large as those hardened by withholding of water, as shown by the relative green weights of A1 and A3 in Table 2. Plants hardened by withholding moisture were usually darker green, covered with heavy waxy bloom, with slight pink tints in the stem and petioles, but not as heavily pigmented as the coldframe hardened plants. The leaves were tough and leathery, in contrast to the brittle, crisp texture of tender plants.

Cabbage plants hardened by exposure in coldframe were smaller and stockier than unhardened greenhouse plants and nearly always showed more or less pink pigment (probably anthocyanin) in the stems, petioles and leaf veins. Coldframe hardened plants were tough and leathery in texture. In most of the experiments the maximum degree of hardening by this method enabled cabbage to withstand a temperature of –5°C. to –6°C. for at least one hour, whereas non-hardened plants would be killed between –3°C. and –4°C. In a few experiments hardened cabbage withstood temperatures as low as –8°C. to –10°C. over night.

The development of pink color, especially in the stems and petioles, was conspicuous in all hardened plants. According to Knudson[55] the "work of Ewart, Overton, Wheldale and others indicates a close relationship between the sugar content of the plant and pigment production." Throughout Knudson's experiments on the effect of carbohydrates on green plants, a tendency to anthocyanin production was observed, plants fed on glucose and maltose (M/20 solutions) showing heavy coloration, which disappeared within a week when they were placed in diffuse light. These results are of special interest in connection with the large sugar content found in hardened plants, discussed later.

Nicholas[91] was of the opinion that "the production (in leaves) of anthocyanin is correlated with the formation of organic acids. The connection known to exist between oxidation and pigmentation inheres in the production of these acids, accompanied by the formation of the red pigment."

The conspicuous development of the waxy bloom on cabbage plants has been considered by Harvey[42] of some importance in relation to cold resistance in that it may permit the undercooling of the

leaf several degrees below the freezing point. He suggests that it prevents the "inoculation" of the moisture in the leaf by droplets of water freezing on the surface.

Cabbage plants hardened by other methods showed much the same changes as did those in the series mentioned above. In all cases hardiness was in direct proportion to the external changes noted. Wherever the growth of the plant was materially checked, even for a few days, hardiness was increased in proportion to the checking.

Cauliflower and kale showed about the same changes on hardening as cabbage.

Leaf lettuce. Both small potted plants and large plants approaching maturity in the greenhouse and coldframe were used. The leaves become tougher, thicker and of more leathery texture upon hardening. Pigmentation was not conspicuous. When hardened by drying, the crinkling of the leaves was more pronounced and the color deeper green.

Tomato. Leaves of hardened plants became very dark green with much pigmentation on the under side, were much smaller, tended to curl on the midrib; the stems and petioles became very heavily pigmented, tough and woody in texture. Hardening tomato plants in the greenhouse by any of the methods of checking growth had about the same effect on external appearance. The same was true of the coldframe-hardened plants, except that when hardening was long-continued at low temperature, the lower leaves turned yellow and fell, until the plant was nearly defoliated. This is probably similar to the form of killing by temperatures above the freezing point noticed by Molisch and attributed by him to the inhibition of metabolism by the low temperatures.

Morphological difference in hardened plants.—Schaffnit[110] was unable to find any structural differences in varieties of wheat varying in degree of cold resistance. Salmon and Fleming[109] could find no difference in cell structure in hardy and tender varieties of cereals. On the other hand, Briggs[16] found that the cells were somewhat smaller in the pistils of hardy varieties of peaches. Walster[124] observed that in barley grown at 15°C., there was greater lignification of the xylem bundles than in plants grown at 20°C. This would make the plants grown at the lower temperature stiffer and stronger.

To determine whether the hardening process affects the size of the cells in vegetable plants, sections were made of hardened and

not hardened cabbage and tomato leaves and the palisade cells measured. Portions of young leaves which had made most of their growth during the process of hardening were used in each case. Hence the differences in the cells here reported do not represent an "acquired" condition, but differences in development between plants under favorable growing conditions and those subjected to hardening. Portions were taken from corresponding locations on leaves of about the same size, killed and fixed in the usual way. Transverse sections were made with the rotary microtone, mounted, stained and measured.

In the tender tomato leaf numerous large air spaces were observed, while in hardened leaves the cells were more compactly arranged and filled with starch grains. Starch grains were less plentiful in hardened cabbage leaves, but the compactness of the cellular arrangement was marked. Table 1 gives the measurements in two dimensions of the palisade cells and the cross-section area computed therefrom. These data are the averages obtained from measurements of several different sections.

TABLE 1.—MEASUREMENTS OF LEAF PALISADE CELLS IN PLANTS HARDENED AND NOT HARDENED.

	Thickness of whole leaf	Thickness of palisade	Thickness of parenchyma	Width of cells	Length of cells	Area, sq. μ.
Cabbage						
Not hardened	291μ	134.5μ	106.3μ	19.1μ	36.3μ	694.0
Hardened by drying in g. h.	269	127.9	106.3	19.4	27.8	538.8
Not hardened	274	136.2	102.1	20.9	36.9	772.0
Hardened in coldframe	312	168.6	118.0	19.9	31.1	619.5
Tomato						
Not hardened	196.6	76.2	76.2	21.0	57.5	1201.5
Hardened	133.7	55.6	68.0	14.1	44.8	630.1

Judging from the data presented in Table 1, hardened plants are characterized by somewhat smaller and more compact palisade cells than are non-hardened leaves of the same sort. In tomato, leaves from plants given hardening treatments are considerably thinner than tender leaves, however, cabbage leaves hardened in coldframe gained in thickness.

Effect of hardening treatments on rate of growth.—The growth of plants subjected to any of the hardening treatments was checked in proportion to the intensity of the treatment. Data are presented in Table 2, on samples gathered from lots of the same age, grown

under otherwise uniform conditions, except for the various hardening treatments. With the average green weight of the plants as the criterion, it is evident that hardened plants are much smaller, indicating the extent to which their growth had been checked. Thus, in the series gathered March 12, 1920, wet-grown greenhouse cabbage plants (tender) averaged 23.1 grams, plants hardened by partial withholding of moisture averaged 6.8 grams and plants hardened in coldframes three weeks averaged 7.67 grams. The differences in dry weight are not so great, since the smaller and hardier plants possessed a larger percentage of dry matter.

In the tomato, the rate of growth could be roughly measured by the increase in height from week to week. Accordingly a number in each of the lots subjected to the various treatments were measured each week. The retardation of growth, when any of the hardening treatments became operative, is shown in figure 1.

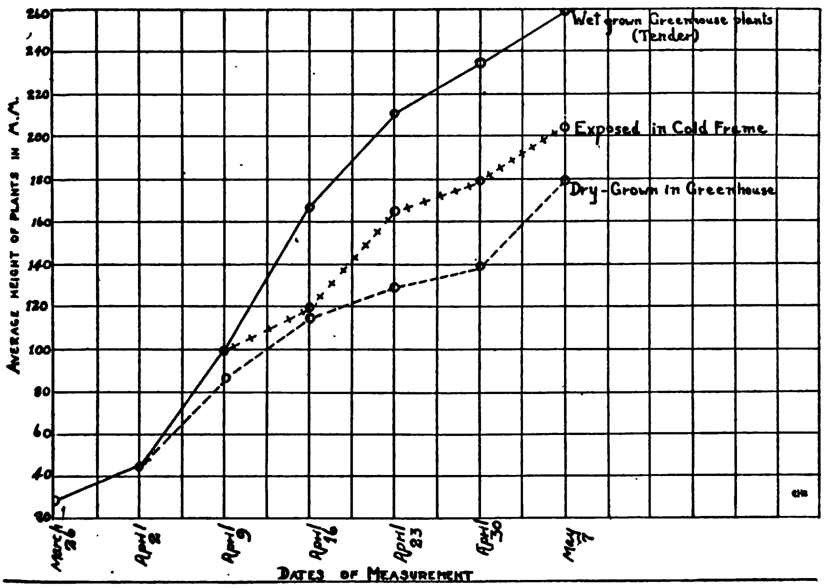

Fig. 1.—Rate of growth of tomato plants under various conditions.

Effect of hardening treatments on percentage of dry matter.—The data given under percent of dry matter in Table 2 indicate considerable increase of dry matter in all of the experimental lots of plants exposed to hardening treatments. Conversely, the water content decreased in hardened plants, roughly in proportion to the extent to which their growth was checked by the hardening treatment.

The possible significance of decreased water content in relation to the water-retaining power of the cell when exposed to water-loss by freezing has already been indicated by Wiegand and more recently by the work of Chandler, Salmon and Fleming, Carrick and indirectly by Parker. It may be repeated here that decreased water content would be associated with increased force of imbibition, and with increased concentration of the cell sap, which forces tend to retain water in the cell during freezing. It is realized that the *total loss* in weight upon drying of leafy tissue does not truly represent the actual water content of the plant, but the difference is probably so small that this loss is taken as the moisture content throughout these experiments.

Effect of hardening treatment on depression of freezing point.— In several experiments, the freezing point depression of the expressed sap of leaves of hardened and non-hardened plants was determined with the usual Beckman's apparatus. Potted plants from each experimental lot were brought into the laboratory to insure having fresh tissue for each determination. All of the leaves were taken from two or three plants and ground. The sap was then squeezed from the macerated pulp and duplicate or triplicate freezing point determinations were made at once. The data given in the column "Depression of the Freezing Point" in Table 2 show that the depression was somewhat greater in the hardened plants, indicating greater osmotic concentration of their sap. Similar data have been obtained by Chandler and by Harvey, working on the same sort of material, hence it was not deemed worth while to make a larger number of these determinations. The differences found here in freezing point depression are somewhat greater than those obtained by Chandler[20] and much greater than those reported by Harvey[42] for hardened and not hardened cabbage. This is due perhaps to the extremes in the treatments used in these experiments. Heavy watering made Series A1 somewhat more tender than ordinary non-hardened plants and Series A3 attained maximum hardiness through the application of the minimum amount of water to keep the plants from wilting.

It may be pointed out that the increased sap concentration in hardened plants is due probably to the combined effect of the following factors: (1) Decreased total moisture content. (See Table 2). (2) Increase in the amount of sap solutes. Numerous investigators have found an increase of soluble sugars in plants exposed to low

TABLE 2.—EFFECT OF HARDENING TREATMENTS ON SIZE OF VEGETABLE PLANTS, PER CENT OF DRY MATTER, DEPRESSION OF THE SAP FREEZING POINT, AND RESISTANCE TO COLD.

Plant	Series No.	Treatment	Date samples taken	Average green wt. plants	Average dry wt. plants	% of dry matter	Depression of freezing point	Relative Hardiness to Cold
Cabbage	A1	Optimum moisture, greenhouse	3/20/19	9.780	0.846	8.65	0.965°C.	
			11/20/19	7.740	0.668	8.6	0.785°C.	Killed at −4°C. in 1 hour
			3/12/20	23.100	1.855	8.2	0.753	Injured at −3° in 2 hours.
	A2	Medium moisture, greenhouse	3/20/19	8.240	0.837	10.17	1.048	Slightly injured at −4° in 2 hours.
			3/12/20	10.05	1.132	11.30	1.115	Uninjured at −3°C. in 2 hours
	A3	Minimum moisture, greenhouse	3/20/19	6.228	0.577	9.22	1.200	Not injured at −4°C. for 2½ hours.
			11/20/19	5.04	0.550	10.90	1.083	Not injured at −3°C. in 2 hours
			3/12/20	6.48	0.830	12.80	1.248	Not injured at −5°C. in 1 hour
	A4	Grown wet at first, then partially wilted for 2 weeks	3/12/20	12.20	1.311	10.76		Uninjured at −3°C. in 2 hours
	E4	Greenhouse plants not hardened	3/12/20	16.68	1.77	10.52		Killed at −4°C. in 2 hours
			4/5/20	20.90	1.956	9.38	1.043	
	E3	Plants hardened in coldframe 1 week	3/21/19	7.787	0.928	11.90	0.963	Uninjured at −4°C. for 1 hour
			11/20/19	5.270	0.602	11.71	0.963	Slightly injured at −4°C. 2½ hours
			3/12/20	13.63	1.680	12.31	1.132	Slightly injured at −6°C. in 2 hours
	E2	In coldframe 2 weeks	3/20/19	7.925	0.894	11.28		Slightly injured at −6°C. in 2 hours
			3/12/20	17.430	2.250	12.91	1.109	
	E1	In coldframe 3 weeks	3/20/19	7.300	0.966	13.24		Slightly injured at −6°C. in 2 hours
			3/12/20	7.670	1.016	13.27	1.157	
	D1	Grown in coldframe	11/20/19	1.195	0.155	12.55	1.200	Not injured at −4°C. in 2½ hours Slightly injured at −4°C. for 1 hr.
	G2	Full light, greenhouse	3/20/19	6.487	0.753	11.60		Not injured at −2°C. for 1 hour
	G3	Light shade, greenhouse	3/20/19	6.580	0.627	9.52		Slightly injured at −2°C. for 1 hr.
	G4	Medium shade, greenhouse	3/20/19	1.654	0.107	6.43		Killed at −2°C. for 1 hour

TABLE 2.—(Continued).

Plant	Series No.	Treatment	Date samples taken	Average green wt. plants	Average dry wt. plants	% of dry matter	Depression of freezing point	
Cabbage	G5	Heavy shade. greenhouse	3/20/19	0.642	0.035	5.46		Killed at –2°C. for 1 hour
	H1	Greenhouse plants severely root pruned	4/5/20	5.58	0.72	12.90		
	H3	Greenhouse plants moderately root bound, not pruned	4/5/20	7.47	0.860	11.50		
	F	Compost soils and tap water	3/30/21	8.12	0.581	7.16		Not injured at –3° in 30 minutes Killed at –6°C. in 30 minutes
		Compost plus $NaNO_3$ M/10	3/30/21	4.63	0.431	9.31		Slightly injured at –6°C. in 30 min.
		Compost plus M/10 KCl	3/30/21	5.38	0.505	9.40		Not injured at –6°C. in 30 minutes
		Compost plus M/10 NaCl	3/30/21	4.76	0.455	9.57		Not injured at –6°C. in 30 minutes
		Compost soil plus manure, plus M/10 KCl	3/30/21	6.18	0.631	10.2		Not injured at –6°C. in 30 minutes
		Compost soil plus M/10 NaCl	3/30/21	6.53	0.624	9.55		Not injured at –6°C. in 30 minutes
		Sand and tap water	3/30/21	3.55	0.967	6.78		Slightly injured at –3°C. in 30 min. Killed at –6° in 30 minutes
		Sand and M/10 $NaNO_3$	3/30/21	8.58	0.560	6.52		Not injured at –3°C. in 30 minutes Killed at –6° in 30 minutes
		Sand plus M/10 KcL	3/30/21	8.32	0.523	6.29		Killed at –6° in 30 minutes
		Sand plus M/10 NaCl	3/30/21	8.56	0.546	6.37		Killed at –6° in 30 minutes

TABLE 2.—(Continued).

Plant	Series No.	Treatment	Date samples taken	Average green wt. plants	Average dry wt. plants	% of dry matter	Depression of freezing point	
Tomato	A1	Optimum moisture, greenhouse plants (leaves only)	5/3/19 9/21/19 12/11/19 5/4/20 4/30/21	36.660 15.083 23.90 49.20 12.96	4.175 1.252 2.016 4.05 1.262	11.40 8.29 8.44 8.24 9.73	0.820°C. 0.815	Killed at −2°C. in 1 hour
	A2	Medium moisture, greenhouse plants (leaves only)	5/3/19 9/21/19 5/4/20 4/30/21	7.811 15.592 24.80 8.85	0.927 1.640 3.050 1.110	11.86 10.52 12.28 12.57	1.450	Severely injured at −2°C. in 2 hrs.
	A3	Minimum moisture, greenhouse plants (leaves only)	5/3/19 9/21/19 12/11/19 5/4/20 4/30/21	2.427 8.641 7.320 11.100 4.83	0.399 0.949 1.100 1.510 0.592	16.45 11.00 15.4 13.6 12.28	1.370 1.588	Killed in 2 hours
	A4	Water partially withheld for 2 weeks	5/4/20	30.90	3.15	10.2	1.533	
	B6	Grown in ¾ loam, ¼ manure, in greenhouse	5/3/19 12/11/19	32.546 46.660	3.878 3.600	11.90 7.72	0.950	Killed at −2°C. in 1 hour
	B4	Grown in garden loam soil	5/3/19 12/11/19	10.149 10.330	1.408 1.000	13.98 9.72	1.018	Severely injured at −2°C. in 1 hour
	B2	Grown in ½ loam, ½ sand	5/3/19 12/11/19	2.448 7.19	0.379 0.771	15.50 10.71	1.180	Uninjured at -2°C. for 1 hour but killed in 2 hours
	B1	Grown in ¼ loam, ¾ sand	5/3/19 12/11/19	2.095 8.120	0.354 0.950	16.90 11.55	1.080	Slightly injured at −2°C. for 1 hour
	E4	Greenhouse plants not hardened	5/3/19 5/4/20	30.715 30.40	3.986 2.88	12.95 9.46	0.725	Killed at −1½°C. in 1 hour
	E3	Hardened in cold-frame for 7 days (leaves only)	5/4/20 4/30/21	27.0 7.61	3.15 0.920	11.67 12.05	0.784	Injured at −2°C. in 1 hour

TABLE 2.—(Continued).

Plant	Series No.	Treatment	Date samples taken	Average green wt. plants	Average dry wt. plants	% of dry matter	Depression of freezing point	
Tomato	E2	Hardened in coldframe 14 days (leaves only)	5/3/19 5/4/20 4/30/21	14.482 14.72 5.54	2.037 1.590 0.736	14.0 10.80 13.23	0.792°C.	Uninjured at –2°C. in 1 hour
	E1	Hardened in coldframe 21 days (leaves only)	5/3/19 5/4/20 4/30/21	14.326 14.50 6.00	1.963 1.440 0.850	13.7 9.93 14.17		Uninjured at –2°C. in 1 hour
Leaf Lettuce	A1	Optimum moisture, greenhouse plants	10/31/19 10/5/20	2.229 24.66	0.124 1.228	5.52 4.98		Killed at –3°C. in 1½ hours
	A3	Minimum moisture, greenhouse plants	10/31/19 10/5/20	1.290 2.47	0.084 0.231	6.56 9.36		Uninjured at –3°C. in 1½ hours Uninjured at –4°C. in 1½ hours
	A4	Minimum moisture, for 2 weeks, greenhouse	10/5/20	6.18	0.519	8.44		Slightly injured at –4°C. in 1½ hrs.
	B6	Rich compost soil, greenhouse	10/31/19	4.487	0.199	5.32		Killed at –3°C. in 3½ hours
	B2	Sandy soil, greenhouse	10/31/19	2.883	0.148	5.13		Slightly injured at –3°C. in 3½ hrs.
	E1	Medium moisture in warm greenhouse	3/24/20	2.00	0.157	7.85		Killed at –4°C. in 2 hours
	E2	Hardened in coldframe for 2 weeks	3/24/20 10/31/19	1.64 1.367	0.173 0.122	10.50 8.80		Uninjured at –3°C. in 3½ hours
	X1	Greenhouse plants nearly mature	11/29/19			3.98		Very tender
	X2	Mature plants in coldframe	11/29/19			7.02		Had been exposed to –5°C. several times with little injury
Head Lettuce		Head lettuce hardened in coldframe 10 days	3/24/20	2.67	0.296	11.13		Uninjured at –4°C. for 2½ hours
Cauliflower		Greenhouse plants not hardened	3/24/20	7.73	0.920	11.9		Killed at –4°C. for 2 hours
		Coldframe hardened	3/24/20	5.42	0.727	13.4		Uninjured at –4°C. for 2 hours

temperature and, as shown later, there is an increased sugar content in hardened vegetable plants. (3) Increased amount of water held in the absorbed state by the cell colloids. Since this absorbed water is probably nearly pure, the sap solutes of hardened plants are held in solution by a smaller volume of water—hence the greater concentration.

Chandler[21] found that a large part of the depression of the freezing point in plant sap was due neither to sugars nor to electrolytes. Recent work by Parker[97] showed that finely divided material in suspension exerted considerable influence on the freezing point and that this depression increases rapidly with decreasing moisture content. Though Parker's work was done with soils and dried, finely ground inorganic colloids, it may be supposed that the organic colloidal particles of plant protoplasm have the same property of depressing the freezing point. Parker attributes the lowering of the freezing point by finely divided material to the force of "adhesion" by which films of the liquid are held on the surface of the solid material. The freezing point depression caused by the finely divided material decreases almost to zero in presence of high moisture content. He explains this by the suggestion that as the amount of liquid increases some of it becomes so far distant from the solid particles and is so weakly held that no depression of the freezing point occurs. This is very nearly the same as Wiegand's theory as to the holding of water in plant cells by "molecular capillarity." It may be that the increase in colloidally-held water alone would account for much of the depression of the freezing point in hardened plants. Therefore it may be said that the apparent increase in osmotic concentration of the sap in hardened plants is merely coincident with the state of being hardy, rather than a cause of it.

EFFECT OF HARDENING ON ICE FORMATION IN PLANTS.

Previous investigations on freezing of plant tissue have shown that water is drawn from the cells to form ice in the intercellular spaces. Some plants are killed once this occurs. Others are capable of withstanding some ice formation, but are killed at lower temperatures. Unhardened cabbage plants are killed by freezing at –3°C. to –4°C. The same plants after being subjected to a hardening treatment, may withstand a temperature of –5°C. to –6°C. or even a few degrees lower.

Müller-Thurgau[74] has shown that by no means all of the water freezes in plant tissue when exposed to temperatures well below

the freezing point. His method of measuring the amount of ice in frozen plant tissue was based on observing the cooling of water in which tissue frozen to a definite temperature was placed, calculating that 80 calories are required to melt one gram of ice. In this way he was able to show that the amount of ice in an apple increased as the degree of freezing increased. The following table is taken from his data on frozen apples:

at	-4.5°C.	percent total water frozen = 63.8
at	-7.3	percent total water frozen = 68.2
at	-8.0	percent total water frozen = 72.4
at	-13.0	percent total water frozen = 74.4
at	-14.8	percent total water frozen = 77.4
at	-15.2	percent total water frozen = 79.3

It appears from these data that the amount of water frozen at each successive fall in temperature decreases fairly regularly until –13°C. is reached, but below that temperature the rate of ice formation increases somewhat. The latter temperatures are far below the killing point for apples, which may affect the results, and Müller-Thurgau's technique may be open to some experimental error.

Müller-Thurgau also made some interesting determinations of the time-rate of ice formation. Kohl-rabi leaves exposed to a temperature gradually declining from –5°C. to –8.25°C. froze at the

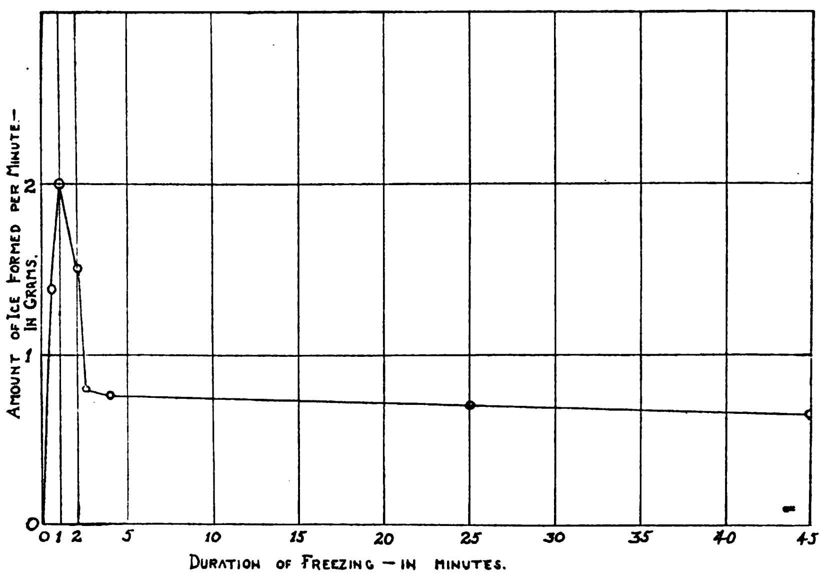

Fig. 2.—Relation of time to rate of ice formation in 100 grams kohl-rabi leaves (arranged from Müller-Thurgau's data).

end of 13 minutes, when the temperature of the leaves was only -4.3°C. and that of the surrounding air was -7.3°C. In the first half minute of freezing 0.69 grams of ice formed in 100 grams of leaves. In the next minute, 2.0 grams, the following two minutes, 1.5 grams per minute, and the next minute, 0.8 grams froze. Thereafter the amount of ice formed per minute gradually decreased until, at the end of one hour of freezing, 41.32 grams of ice had formed in 100 grams of leaves, probably a little over 50 per cent of the total water content. This experiment is illuminating as to the relation of the time factor to freezing of plants. It has been observed that in plants, such as kohl-rabi, which can withstand some ice formation, injury at a temperature near the death point is proportional to the duration of exposure. This fact has been observed frequently in experiments here and Harvey[42] presents in his paper an excellent series of photographs illustrating the same thing. Figure 2 plotted from Müller-Thurgau's data, illustrates graphically the rate of ice formation. We may regard the progressive increase in total amount of ice and the decrease in amount frozen per minute as being due to the balanced action of the force of crystallization and the water-retaining power of the cells, the rate of ice-formation approaching zero as a limit.

Foote and Saxton[28] used the dilatometer in experiments on the freezing of inorganic colloids and were able to show that in such materials water existed in three forms, viz., free water, capillary absorbed water and water of chemical combination. Recently, Bouyoucos[10] and McCool and Millar have made use of the dilatometer to measure the amount of water freezing in soils, in plants and in seeds. McCool and Millar in their latest report, state that the amount of water freezing in plants at -1.5°C. decreased as the concentration of the sap increased (as measured by the freezing point method). At -4°C. the amount of water freezing was considerably more than at -1.5°C. and its correlation with the freezing point of the sap almost disappeared. The following figures rearranged from their report illustrate this:

Crop-plant	Date	Freezing point depression	Amount of water freezing in 5 grams of leaves. At -1.5°C.	At -4°C.
Wheat	Nov. 14	1.107°C.	0.40cc.	2.65cc.
Rye	May 17	1.030	.86	2.40
Rye	Nov. 24	.928	.90	2.50
Sweet clover	Nov. 24	.906	1.22	2.82
Red clover	May 15	.780	1.70	2.70
Corn	June 10	.578	2.10	2.90

Unfortunately they did not express their results as percentages of the total moisture content in each of the different plant tissues, hence it is impossible to draw very definite conclusions. Also nothing is stated as to the source of the material, whether greenhouse or field grown. One interesting point to be noted here is that wheat and rye, which one would expect to be more cold-resistant than corn or clover, show a smaller amount of water frozen at –1.5°C. and somewhat less at –4°C. It is rather surprising that such decided variation in sap density made so little difference in the amount of water freezing at –4°C.

In another series of experiments with corn and barley McCool and Millar[80] showed that varying the soil moisture content affected the water relations in the plants. Freezing point depression and the percentage of moisture in the tops decreased slightly in plants grown with 15.53 percent soil moisture, as compared to those grown with 23.29 percent soil moisture. The amount of water freezing at –2.5°C. was decreased somewhat, and the amount freezing at –4.5°C. was decreased considerably in plants grown on the soils of lower moisture content.

Hibbard and Harrington[45] found that the freezing point of the sap of roots and tops of corn plants fell regularly as the moisture content of the soil in which the plants were grown was decreased. The following data from their work illustrate the relation of soil moisture to freezing point depression of sap.

Percent moisture in soil	Freezing point depression of tops	of roots
31	1.835°C.	.492°C.
23	1.920	.600
16	2.027	.647
13	2.120	.942
11	2.204	.995

McCool and Millar[80] studied the effects of varying the concentration of the soil solution, when the moisture content was kept constant. They found a progressive increase in the freezing point depression of the tops and roots of plants grown in the greater concentrations, but the amounts of freezable water showed little variation. Earlier experiments by the same authors[78] showed that the freezing point depression of both tops and roots varied in the same direction as the concentration of the soil solution in which the plants were grown but not in proportion to it. They also studied the effect of varying the soil moisture content, keeping the concentration of the soil solution constant. Unfortunately, in their experiment

the plants on the high moisture soils took up a larger quantity of the nutrient salts so that the concentration of the soil solution soon became less than in the low moisture soils. Therefore, it remains undetermined whether the effects of wet and of dry soils on freezing point depression and amount of freezable water in plants grown thereon are due to the variation in the water supply, or to variation in concentration of the soil which is involved, or to both.

Method of measuring amount of water freezing in plant tissues. Though Müller-Thurgau was able to obtain considerable data on this subject by measuring the latent heat of ice in frozen tissues, his method is laborious and perhaps open to some experimental error. The dilatometer method, as described by Bouyoucos[11], presents great advantages in its directness, simplicity, and accuracy for measurements at different degrees of freezing. The use of the dilatometer is based on the fact that one gram of water increases approximately one tenth of its volume upon freezing. It has been used in this work essentially as described by Bouyoucos, and by McCool and Millar.

A definite weight (4–6 grams) of fresh leaves, is placed in the bowl of the dilatometer, which is then filled with petroleum ether (boiling point 63°C.). The dilatometer is then stoppered with a rubber cork through which a thermometer is placed, so that the bulb is in contact with the leaf tissue and the scale convenient for reading. It was found at the beginning of this work that quicker and more accurate results can be obtained with plant tissues, by placing the loaded dilatometer in crushed ice for 15 to 20 minutes, to lower the temperature of the whole mass slowly and evenly to the neighborhood of 0°C. After this preliminary cooling, the dilatometer is plunged into an ice and salt bath, mixed in such proportions that the temperature is slightly below that which is desired in the dilatometer. Usually the temperature of the plant tissue in the dilatometer lags slightly above that of the freezing mixture. The dilatometer must be kept perfectly still after it is placed in the bath in order to secure uniform under-cooling of the plant tissue, but the freezing mixture can be gently stirred so as to keep all parts of the bath at uniform temperature.

At first the column of petroleum ether in the graduated side-arm of the dilatometer falls rapidly due to the contraction of the contents on cooling. It is usually necessary to add a little more ether to bring the column up to the point on the scale where it can

be read easily. When the thermometer indicates that the contents of the dilatometer have been desired temperature for several minutes, the position of the column in the side-arm is read, then solidification of the under cooled water in the plant tissue is caused by tapping the dilatometer against the sides of the bath. As solidification of the water takes place, the column rises in the side arm, slowly at first, then more rapidly and then quite slowly for several minutes. It usually takes 5 to 10 minutes to establish equilibrium, indicating that all the water is frozen which will freeze at that temperature. When the column of ether in the side arm becomes stationary, the reading is taken. The amount of expansion as a result of the freezing is the difference between the readings before and after freezing. In this work the side arm tube was graduated to 0.01 cc. and readings could be made to 0.005 cc. The expansion on freezing multiplied by 10 gives the number of cc. of ice formed. In some of the earlier experiments separate samples were used for moisture and dry matter determinations. Later it was found that these determinations could be made on the dilatometer sample after the freezing experiments had been performed. The water content of the tissues being known, the percentage of the total water frozen can be calculated by dividing the number of cc. of ice formed by the total water content of the sample.

Effect of temperature on amount of water freezing in hardened and non-hardened cabbage leaves.—Cabbage leaves were used in most of the experiments, since these were available in varying degrees of hardiness. It was found very difficult to secure rapid crystallization in hardened cabbage leaves at a temperature higher than –3°C., so that point was taken for the minimum reading. On the other hand, leaves could seldom be under cooled below –6°C. without ice formation, so that point was taken as the maximum limit of freezing in most of the experiments. Dilatometer determinations were made a number of times with each class of material under experiment. These determinations were distributed over a period of several months. The samples were taken at different times of day, but in each series samples were taken at the same time of all the different types of material which were being compared. Table 3 gives the results secured with leaves of non-hardened greenhouse cabbage plants thoroughly hardened in coldframe (9-12 days) and of plants hardened in the greenhouse by partial withholding of water for two weeks or more. Each figure represents an average of several determinations, the individual determinations sometimes varying as much

as 10 percent due to slight differences in the material and to the hour at which the samples were taken.

TABLE 3.—PERCENTAGE OF TOTAL MOISTURE IN CABBAGE LEAVES FROZEN AT DIFFERENT TEMPERATURES.

Previous treatment of plants	Percent of dry matter	Percent of total moisture freezing at			
		-3°C.	-4°C.	-5°C.	-6°C.
Wet-grown greenhouse plants, (tender)	10.29	49.9	75.2	82.1	84.2
Dry-grown greenhouse plants, (hardy)	12.7	27.2	48.9	62.6	71.0
Hardened in coldframe 9-14 days, (hardy)	14.67	29.8	49.6	58.7	64.3

Considerably more water froze in the tender plants than in the hardened at each temperature. The material used for the dry-grown hardy plant determinations perhaps was not quite so uniformly hardy in its nature as that of the two other types. The outstanding feature is the progressive increase in percentage of total moisture frozen as the temperature is lowered. This is brought out clearly in Figure 3 plotted from the data in Table 3. The increase becomes less and less for each degree of temperature lowering. Thus in the case of the tender cabbage, 26.3 percent more water freezes at -4°C. than at -3°C., 6.9 percent more freezes at -5°C. than

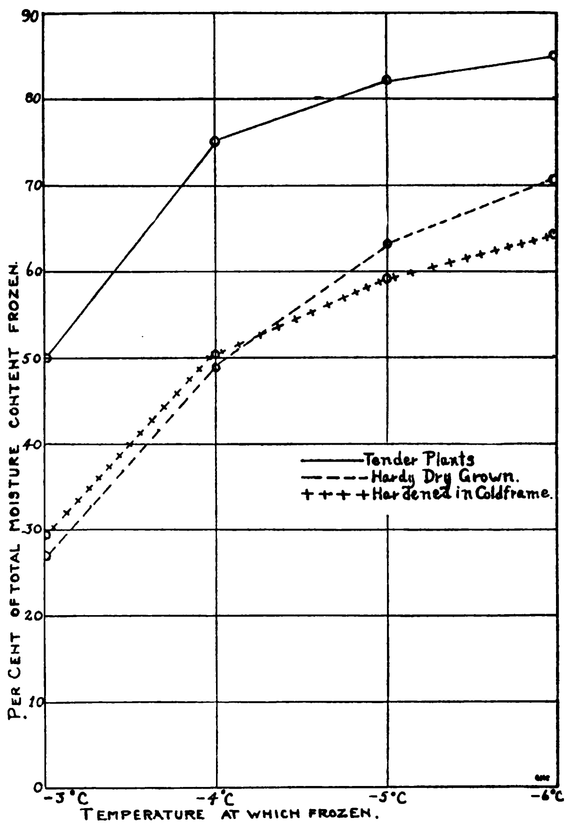

Fig. 3.—Relation of temperature to percent of total moisture freezing in tender and hardened cabbage leaves.

at -4°C. and 2.1 percent more freezes at -6°C. than at -5°C. In the coldframe hardened plants, 19.8 percent more water is frozen at -4°C. than at -3°C., 9.1 percent more at -5°C. than -4°C., and 5.6 percent more at -6°C. than at -5°C. Table 3 shows that the percentage of total moisture frozen at a given temperature is less in the hardened plants. Table 3A, constructed from the same data, on the basis of 100 grams fresh tissue, shows that the actual amount of water remaining unfrozen is greater also in the hardened leaves although there is a smaller total moisture content in such tissues than in tender leaves.

TABLE 3A.—AMOUNT OF WATER IN 100 GRAMS OF CABBAGE LEAVES REMAINING UNFROZEN AT DIFFERENT TEMPERATURES.

Treatment	Percent dry matter	Percent moisture	Grams water remaining unfrozen at			
			-3°C.	-4°C.	-5°C.	-6°C.
Wet-grown greenhouse plants, tender	10.29	89.71	34.9	22.3	16.1	14.3
Dry-grown greenhouse plants, hardy	12.70	87.30	63.5	44.6	32.6	25.3
Coldframe, hardened for 9-14 days	14.67	85.33	59.9	42.9	35.2	30.4

Since the percentage of the total moisture which freezes at each temperature is materially less in hardy than in tender plants, and since the actual amount of water remaining unfrozen is greater in the hardy than in the tender plants, we may safely assume that the cells of the hardened plants possess a greater power to retain water when exposed to freezing. Although the amount of water frozen increases with the lowering of the temperature, we may assume that whatever the nature of the water-retaining force, it is overcome in successively smaller increments by the force of crystallization as the temperature is lowered. The percentage of water remaining unfrozen in the hardened leaves is approximately a logarithmic function of the temperature.

The hardiest plants used in this experiment probably could have been killed by long exposure to -6°C. to -8°C. However, it may be predicted from the rate of increase in the amount of water frozen at the lower temperatures, that if in some way the water-retaining power of the cells in these plants was increased slightly, a much lower temperature could have been sustained. Maximow has shown that sections of cabbage leaves which were injured at -5.2°C. when

frozen in water, successfully withstood a temperature of –32°C. in 2-mol. sugar solution.

Changes in amount of freezable water during the hardening process.—Harvey[42] stated that cabbage plants kept at 3°C. for 24 hours showed slightly increased hardiness and at the end of five days a considerable degree of hardiness was developed. In the present experiments, absolutely controlled conditions were not available; however, it is generally considered that about two weeks' exposure of greenhouse-grown cabbage plants in the open coldframe during March will bring about maximum hardening. To study the relation of the amount of freezable water to the hardening process, lots of tender cabbage plants were removed from the greenhouse to the coldframe at intervals and dilatometer determinations made on the leaves of these plants which represented progressive degrees of hardening. The results are presented in Table 4.

TABLE 4.—AMOUNT OF WATER FREEZING AT -5°C. IN CABBAGE LEAVES HARDENED IN COLDFRAME FOR DIFFERENT LENGTHS OF TIME.

Treatment	Percent dry matter	Percent water	Percent total water frozen	In 100 grams of tissue	
				grams water frozen	grams water unfrozen
Not hardened	9.91	90.09	82.1	73.96	16.13
Hardened 2 days	13.20	86.80	75.3	65.32	21.48
Hardened 4 days	13.90	86.10	62.8	54.47	31.63
Hardened 9 days	14.00	86.00	58.7	50.48	35.62
Hardened 14 days ...	14.79	85.21	54.6	46.52	38.69
Hardened 16 days ...	18.7	81.3	51.0	41.46	39.84
Hardened 20 days ...	19.35	80.65	47.9	38.63	42.02

It appears that the percentage of the total water frozen at –5°C. decreases as the plant tissue increases in hardiness. At the same time the amount of total moisture in the plants decreases, accompanied by an increasing percentage of dry matter. The relation of degree of hardening to the percentage of freezable water and to dry matter content is shown graphically in Figure 4. The dates of decrease in percentage of water frozen and of increase in percentage of dry matter proceed quite rapidly the first four or five days the plants are exposed to hardening in the coldframe. After this, these changes proceed slowly for some days longer. On the whole, it seems that there is a close correlation between the degree of hardiness and the percentage of total water retained in the unfrozen condition. The actual amount of ice per gram of fresh leaf tissue also decreases

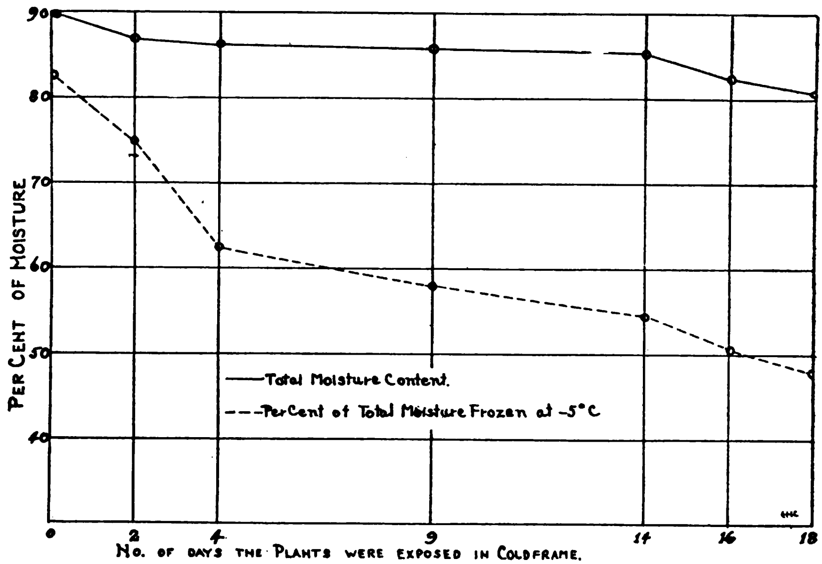

Fig. 4.—Relation of degree of hardiness to percentage of total moisture and percentage of water at -5°C. in cabbage leaves.

with the degree of hardening, while the actual amount of water remaining unfrozen increases as shown in Figure 5.

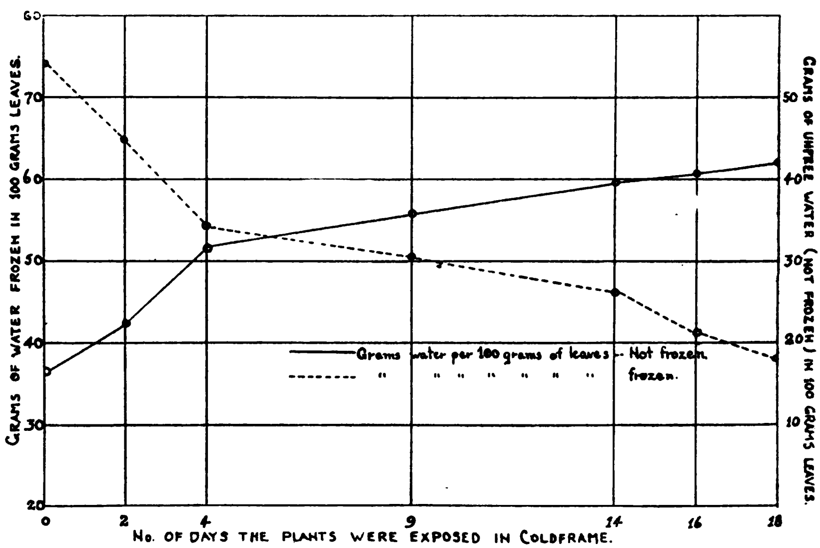

Fig. 5.—Relative amounts of water frozen and not frozen at -5°C., in 100 grams cabbage leaves of varying degrees of hardiness.

Influence of time of day on percentage of water frozen.—McCool and Millar[80] found that the time of day influenced both the depression of the freezing point and the amount of water frozen at a given temperature. Their experiments with various cereal plants showed that the freezing point depression of the leaves increases during the forenoon, declines slightly in the afternoon and almost reaches the early morning value by midnight. Over the same period the percentage of total moisture varied inversely with the depression of the freezing point, but to a much less degree. Shaded oat plants decreased steadily in sap density during the day, while exposed plants showed the usual increase at mid-day. The slight difference in water content of plants is held by these writers to be insufficient to explain fully the increased sap concentration at mid-day, hence it seems that the products of photosynthesis must play a part. Barley plants kept under bell jars in a saturated atmosphere, under conditions retarding transpiration but permitting photosynthesis, had 55 percent of the water in the tops and 62 percent of that in the roots frozen at –3°C. to –4°C. in the morning. At noon 43 percent of the water was frozen in the tops and 59 percent in the roots, at the same temperature.

It has been shown by Dixon[183] that illumination increased the osmotic concentration in leaves and this concentration gradually fell when light was cut off. Chandler[20] also found that plants shaded 24 hours had decreased concentration. According to Drabble and Drabble,[184] a greater concentration of cell sap occurs in plants subjected to factors favoring rapid loss of water by transpiration. Under these conditions the increased concentration of cell sap is probably very largely the result of, as well as the means of protection against, rapid loss of water from the leaves.

In the course of the experiments on the amount of water freezing in cabbage leaves of different degrees of hardiness, some data were obtained relative to the effect of the time of day on the amount of water freezing in leaves of the same hardiness. No attempt was made to provide specially controlled conditions; they were the same as those previously described for the various hardening treatments and were identical with those referred to in Table 2.

TABLE 5.—EFFECT OF TIME OF DAY ON AMOUNT OF WATER FROZEN IN CABBAGE LEAVES AT -5°C.

Material	Time	Percent moisture in plants	Percent water frozen at -5°C.
Wet-grown greenhouse plants (tender)	9 A. M.	90.43	82.4
	2 P. M.	90.22	78.2
	6 P. M.		85.9
Dry-grown greenhouse plants (hardy)	9 A. M.	86.60	55.8
	2 P. M.	86.39	47.1
Coldframe hardened plants	9 A. M.	87.87	61.9
	2 P. M.	84.12	55.5

It is seen that the amount of freezable water is somewhat greater in the morning than in early afternoon, but the differences are not as great as those found by McCool and Millar. The moisture content is also somewhat less in the afternoon indicating the possibility of a greater power of imbibition at that time. Probably the larger factor in causing the slight difference in amount of frozen water is the increased concentration of sugars formed by the photosynthetic activities of the leaf. Both the moisture content and the concentration of cell sap evidently have some influence on the amount of freezable water.

Effect of watering plants with salt solutions on amount of easily frozen water in the leaves.—A method used to harden vegetable plants was watering with salt solutions. Only one of these experiments will be discussed here. On February 15, seedling cabbage plants were potted in 3-inch clay pots, which were plunged in soil on a raised bench in the greenhouse. One series was potted in river sand, one in greenhouse compost soil and a third in compost plus rotten stable manure. Each series was divided into four plots and after the plants were well established one of each series was watered with: (1) tap water, (2) M/10 $NaNO_3$, (3) M/10 KCl, (4) M/10 NaCl. These applications were repeated every few days, when water appeared to be required. After the second application, the rate of growth in the different plots was evidently being affected. All of the salt solutions depressed growth but particularly in the series grown in compost and in the compost and manure mixture. Plants growing in the sand showed some of this stunting effect, but much later than in compost soils. A test made March 30 showed that the plants grown in the compost soils and stunted by the salt applications were much hardier than those receiving tap water and making normal growth. Little effect of the salts upon either the size or

hardiness of the plants grown in sand could be observed. Plants grown in the compost soils and watered with NaCl were exposed to -6°C. for 45 minutes without injury. Plants in compost soils watered with KCl and $NaNO_3$ were injured somewhat under the same conditions, and those receiving tap water were killed. Plants from all of the lots grown in sand were killed at -6°C., but when exposed to -3°C. to -4°C. for one hour, only those receiving water were much injured. The day following the freezing tests, dilatometer determinations were made on leaves of plants from some of the lots given different treatments, the results being shown in Table 6. Most of these figures represent only one determination. The samples were gathered about 1:30 P. M. on a bright sunny day, which may explain why the percentage of water frozen in some of these plants is a little less than that shown in Table 3 for tissues of approximately the same degree of hardiness.

TABLE 6.—AMOUNT OF WATER FREEZING AT -5°C. IN CABBAGE LEAVES FROM PLANTS WATERED WITH VARIOUS SALT SOLUTIONS.

Treatment of plants	Percent dry matter	Percent moisture	Percent total water frozen at -5°C.	Grams water frozen in 100 grams of leaves
In compost soil watered with tap water (medium tender)	10.86	89.14	61.2	54.55
In compost soil watered with M/10 $NaNO_3$ (hardy)	11.83	88.13	37.3	32.87
In compost soil watered with M/10 NaCl (hardy)	12.02	87.98	39.5	34.76
In compost and manure-watered with M/10 NaCl (very hardy)	14.03	85.97	27.2	23.29
In sand watered with tap water (very tender)	8.24	91.76	79.8	73.22
In sand watered with M/10 NaCl (medium tender)	11.01	88.99	59.4	52.86

The percentage of water frozen is much less in the stunted plants—those found most hardy to cold. The amount of water frozen is correlated with the observed degree of cold-resistance and the extent to which growth was checked. Here again the percentage of water frozen varies inversely with the percentage of dry matter. Unfortunately the freezing point depressions of the plants used in this experiment were not taken. However, we know from Chandler's work that the sap of plants watered with salt solutions has an increased osmotic concentration. Bartetzko[4] found that *Aspergillus*, *Penicillium* and other fungi grown in nutrient media of varying

concentrations increased their resistance to freezing in proportion to the increase in the osmotic strength of the medium.

The question arises as to how the application of salt solutions to soils in which plants are growing checks growth, increases cold resistance and reduces the amount of freezable water. The stunting might be due to: (a) The toxicity of the salt solution to the roots of the plants at the concentration used. However, since the salt solutions were not appreciably toxic to the plants grown in sand, it seems doubtful if the stunting and hardening of the cabbage plants in the compost soils can be attributed to this factor. (b) Absorption of the salts by the plants, causing a greater concentration of the sap, yet why should nutrient salts such as $NaNO_3$ cause a stunting of healthy plants? (c) Condition of physiological drought within the plant, at least at such times as the moisture content of the soil was low or the rate of transpiration very rapid. Such a condition might easily arise, in treating a succulent plant such as cabbage, with rather strong salt solutions. If the tops of the plants suffered from physiological drought a considerable part of the time because the roots were unable to absorb water rapidly from the more concentrated soil solution, then a condition would exist more or less similar to that in ordinary soils wherein plants have been hardened by partially withholding moisture. The fact that the lots grown in sand did not show nearly so much of the checking or stunting effect as those grown in the finer soils containing more organic matter lends strength to this idea. To see whether or not the observed results might be due to variations in the concentration of the soil solution this was determined in each lot at the end of the experiment. The method of Bouyoucos[18] was employed, taking 15 grams of air-dry soil and 10 cc. of distilled water, determining the freezing point depression with the Beckman thermometer, and calculating that the soil solution contained 100 parts of solute per million for each 0.004°C. of freezing point lowering. The results are presented in Table 7.

TABLE 7.—CONCENTRATION OF SOIL SOLUTIONS AFTER M/10 SALT SOLUTIONS WERE APPLIED FIVE TIMES.

Treatment	Sand		Compost		Compost and manure	
	Freezing point depression	p.p.m. in soil solution	Freezing point depression	p.p.m. in soil solution	Freezing point depression	p.p.m. in soil solution
Tap water ..	.005°C.	125	.045°C.	1125	.159°C.	3975
M/10 $NaNO_3$ ..	.020°C.	400	.277	6925		
M/10 KC1 ..	.020°C.	400	.210	5200		
M/10 NaCl ..	.020°C.	400	.263	6576	.367	9175

The results set forth in Table 7 show that at the conclusion of the experiment, just after samples for the dilatometer determinations had been taken, the concentration of the soil solutions had increased very markedly in the compost soils to which the salts were applied, as compared to the check of the same sort of soil, but receiving tap water. However, in sand the soil solution was much less concentrated and there was no great increase in the concentration of the soil solutions where the salts were applied. Bouyoucos[13] has shown that

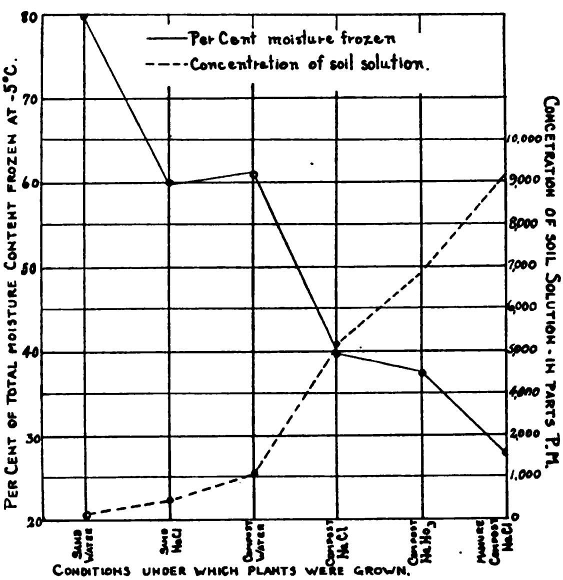

Fig. 6.—Relation of soil solution concentration to percent of water freezing at -5°C. in cabbage leaves.

soils containing much organic matter cause a large amount of practically pure water to become "unfree" by means of capillary adsorption. At a given moisture content therefore, the free solution in such soils would be more concentrated than in a sandy soil having little organic matter and large soil particles. Comparing the data in Tables 6 and 7 it is observed that the amount of water frozen in plants at -5°C. varies inversely to the concentration of the soil solution, but probably not in proportion to it. This point is illustrated by Figure 6.

To show to what extent the growth of plants in this experiment was affected by the salt applications in the three soil media used, the average green weights, average dry weights, and percentages of dry matter are given in Table 8, for the plants in each lot at the end of the experiment (one day after the freezing determinations were made).

TABLE 8.—AVERAGE GROWTH MADE BY CABBAGE PLANTS IN SOILS RECEIVING FIVE APPLICATIONS OF M/10 SALT SOLUTIONS.

Treatment	Sand			Sandy compost			Sandy compost and horse manure		
	Green wt.	Dry wt.	% dry matter	Green wt.	Dry wt.	% dry matter	Green wt.	dry wt.	% dry matter
Tap water ...	13.55	.967	6.78	8.12	.581	7.16			
M/10 $NaNO_3$.	8.58	.560	6.52	4.63	.431	9.31			
M/10 KCl ..	8.32	.523	6.29	5.38	.505	9.40	6.18	.631	10.22
M/10 NaCl ..	8.56	.546	6.37	4.76	.455	9.57	5.53	.624	9.55

It is seen from Table 8 that the plants in the sand made nearly twice as much growth as plants receiving corresponding treatment in the compost soils, using the average green weights as the indicator. It should be remembered that in this experiment a rather high soil moisture was maintained in order to prevent the lack of water from influencing the results; furthermore this experiment was ended before the lack of nutrient material in the sandy soils could become the main factor limiting their growth.

The indications point to the conclusion that applications of rather strong salt solutions raised the concentration of the soil solution to a point at which roots could take up water only slowly, and probably not at all when the total soil moisture content fell below a certain point. This developed a state of physiological drought in the tops due to the restricted water intake. Under these conditions, the leaves developed xerophytic characteristics to some extent, as indi-

cated by the greatly increased water-retaining power on the part of the cells. This is shown by the smaller amounts of water frozen in the leaves of such plants, and as is shown later, by the lower transpiration rate, and slower rate of drying in an oven. Another example of increased cold resistance apparently resulting from physiological drought was observed in the field in the spring of 1921. On March 31, the temperature fell to –8°C., and the following night to –6°C. Hardened cabbage plants set in the field 10 days previous, were very severely injured, but here and there thru the field small plants were observed after the freeze, the leaves of which were apparently uninjured. On examination, the stems of all such plants were found to be nearly severed by a "damping off" fungus. Evidently the stem injury by the fungus had caused physiological drought in the top of the plant, resulting in considerable increase in hardiness.

Relation of amount of freezable water to percentage of dry matter and freezing point depression in garden plants.—Three species of plants were used in these experiments, cauliflower representing a group possessing potential hardiness, and tomatoes and sweet potatoes representing plants lacking potential hardiness. Leaves were gathered during June from plants growing under ordinary conditions in the garden. The soil was fairly moist at this time and the plants were making good growth. A portion of each lot of leaves was used for the dilatometer determination, and another portion for determination of the freezing point depression. This latter was made, not on the expressed sap, as were the previous determinations herein reported, but directly on the triturated leaf tissue, according to the method of Bouyoucos and McCool.[14] The results are given for two sets of determinations in Table 9. In the last two columns of this table are given the relative amounts of frozen and unfrozen water, calculated on the basis of 100 grams of fresh leaf tissue.

The plants used in these experiments would probably have been killed by a brief exposure to –3°C., except the cauliflower, which might have withstood a somewhat lower temperature. It may be seen from Table 9 that the percentage of total water freezing in cauliflower at –5°C. is somewhat less than in tomato and sweet potato, while at –3°C. this difference is much greater in favor of the hardier cauliflower. The amount of water remaining unfrozen is correspondingly greater in cauliflower. It appears that allowing cauliflower leaves to stand in 8 percent sucrose over night has increased the percentage of dry matter and the freezing point depression and has decreased the amount of water freezing at –5°C. The

TABLE 9.—AMOUNT OF WATER FROZEN IN LEAVES OF GARDEN PLANTS.

Date	Plant	% dry matter	Freezing point depression	In 100 grams leaf tissue frozen at -5°C. % water frozen	grams water frozen	grams water unfrozen
June 8	Cauliflower (in 8% sucrose over night)	15.86	.780°C.	57.0	48.0	36.14
" "	Cauliflower (in water over night)	13.26	.413	77.4	67.1	19.64
" "	Tomato	11.73	.650	79.3	70.0	18.27
" "	Sweet Potato	17.5		83.0	69.4	13.1
				Frozen at -3°C.		
June 21	Cauliflower	17.7	1.300	28.3	23.3	59.0
" "	Tomato	13.5	.915	43.7	37.9	48.6
" "	Sweet Potato	14.84	.750	48.1	41.0	44.16

amount of water remaining unfrozen in the cauliflower leaves which had been in the sugar solution is nearly twice as much as in the check leaves kept in water. Since sucrose penetrates plant tissue quite slowly, the changes noted are probably not due to increased sugar content. However, the dry matter is 2.60 percent or nearly $\frac{1}{5}$ greater in the leaves placed in sugar solution. The 8 percent sucrose solution is approximately equivalent to 0.25 molecular concentration. Under these conditions, water may be withdrawn from the leaf, thereby decreasing the moisture content, increasing the percentage of dry matter and presumably increasing the power of imbibition with which the remaining moisture is held by the leaf cells. Rather tender cabbage plants, the roots of which were placed in 8 percent sucrose solution, wilted quickly, indicating withdrawal of water from the upper portion of the plant, or at least stoppage of intake to make up losses by transpiration.

Resumé.—It does not necessarily follow from the water-loss theory of killing by cold that there is a definite minimum moisture content below which the protoplasm of all plants dies. In view of experiments such as those of Adams[3] and of Kiesselbach and Ratcliff[52] it seems quite likely that the minimum amount of water required by plant cells to retain life varies with the state of physiological activity, the stage of development, perhaps with changes in either internal or external conditions, and probably differs in various species at the same stage of development and under the same conditions. Ewart[135] has shown that some seeds can be dried to a moisture con-

tent of 1 or 2 percent without killing and there is reason to believe that if a tender cabbage leaf is killed by the loss of 50 percent of its water, a hardened leaf may be able to survive the loss of even a larger fraction at still lower temperatures. May not the hardening process in vegetable plants, the maturing process in woody stems and the ripening process in seeds involve changes which increase the stability of the protoplasmic structure as well as changes which make for increased water-retaining power?

RATE OF WATER-LOSS BY TRANSPIRATION IN HARDENED AND TENDER CABBAGE.

It is a commonly observed fact that non-hardened vegetable plants wilt severely upon transplanting to the field and if conditions favor rapid transpiration or if the soil is dry they may die, due to excessive water loss. On the other hand, plants properly hardened by any of the methods mentioned in this paper withstand transplanting without serious wilting. To the practical grower the ability of hardened plants to survive transplanting without dangerous wilting is probably of greater importance than the increased cold-resistance developed by the hardening process. Plate 6, B and C, illustrates the marked difference in turgor of hardened and not hardened cabbage plants one day after transplanting to the field. These were potted plants, so the root systems were not disturbed much by transplanting.

Of interest in this connection are the observations of Bergen[7] on the rate of transpiration of a number of evergreens, as *Olea, Quercus* and *Pistacia,* compared to that of *Ulmus* and *Pisum sativum.* He found that the water loss in the former group was 25 percent less than in the latter. He concluded, however, that xerophytic leaf structure (of the hardy evergreens) is not always incompatible with abundant transpiration, but sometimes exists only for use in emergencies, to protect the plant from injurious loss of water.

Salmon[108] draws attention to the xerophytic structure of the hardiest types of winter cereals; winter rye, Turkey and Kharkoff wheats are characterized, for example, by a narrow leaf and prostrate habit of growth. The same is true of Winter Turf, the hardiest variety of winter oats. Salmon found no differences in cell structure, epidermal covering, or mechanical ability to control transpiration, that could be correlated with the great difference in hardiness known to exist in cereals, except that Turkey wheat (hardy) had 25 percent greater root length than Fultz (less hardy) and 40 percent greater than oats and barley (least hardy). This character might enable the

plant to escape dangerous drying out when the ground is frozen to a certain depth.

The relation existing between water-retaining power and resistance to cold is demonstrated by observations of workers[53] in the United States Forest Service, in a recent study of a chlorosis of conifer seedings. The chlorotic leaves were less turgid than normal leaves and wilted very quickly when the water supply was cut off; in fact, chlorotic leaves of the Douglass fir wilted so quickly that accurate leaf measurements could not be made. Plants having chlorotic leaves failed to harden properly in the fall, so that many were injured by early fall frosts and many more by winter cold. However, in plots where the chlorosis was corrected in summer by spraying with ferrous sulfate, the plants became perfectly winter-hardy. Evidently chlorotic leaves are unable, because of absence of chlorophyl, to develop the usual water-retaining power and cold resistance of the species.

It was considered desirable to determine the difference in rate of transpiration of non-hardened plants and plants hardened in various ways, because of the indications which might be obtained thereby as to the relative water-retaining power of plants of different degrees of hardiness. Four experiments were performed, using cabbage plants in 4-inch clay pots. The pots were coated and sealed with a mixture of paraffin, vaseline and beeswax. Two to four plants were used from each experimental lot. Before sealing, the pots were brought to uniform moisture content. The experiments were conducted under different conditions, but in each experiment the plants were kept uniform with reference to external factors. Plants as nearly the same size as possible were used, but the hardened plants were usually smaller than the non-hardened. At the conclusion of each experiment the plants were weighed at once and the leaf area of each plant was measured with a planimeter. The results of the four experiments are presented in Table 10.

TABLE 10.—TRANSPIRATION EXPERIMENTS WITH CABBAGE PLANTS.

Treatment of plants	No. plants used	Av. leaf area per plant	Av. Amt. transpired per plant	Transpiration in grams per hour: per plant	Transpiration in grams per hour: per sq. M. leaf area
Expt. 1 (outdoors) 3/15/21 partly cloudy, cool, moderate wind, 24 hours					
Dry-grown gh. plants	4	125.0 sq. cm.	13.15 g.	0.547	43.7
Wet-grown gh. plants	2	354.0	39.6	1.649	46.6
Expt. 2 (In cool greenhouse) 3/15/21 temp. 60–70 degrees F., 24 hours					
Dry-grown gh. plants	4	167.0	12.95	0.539	32.3
Wet-grown gh. plants	2	285.0	27.9	1.162	40.8
Expt. 3 (In warm greenhouse) 3/19/21 temp. 65–80 degrees F., 24 hours					
Coldframe hardened for 5 days	2	347.0	34.60	1.441	41.5
Dry-grown gh. plants	4	165.0	19.22	0.800	48.5
Wet-grown gh. plants	2	315.0	41.15	1.714	54.4
Expt. 4 (Outdoors) 4/2/21, clear, warm, little wind, 5 hours, 11:30 A. M. to 4:30 P. M.					
Coldframe hardened for 1 week	2	278.3	18.90	3.778	135.6
Med. dry-grown gh. plants	3	202.0	12.97	2.590	128.3
Med. wet-grown gh. plants	2	395.5	29.6	5.920	150.0
Greenhouse plants grown in compost soil and watered with M/10 NaCl (hardy)	2	153.6	10.5	2.100	136.6
Same, watered with tap water (tender)	2	164.0	15.9	3.180	193.9
Greenhouse plants grown in sand, and watered with tap water (tender)	2	252.8	23.9	4.780	189.0

The water loss per square centimeter of leaf area per hour is somewhat greater in tender plants than in those hardened by drying, by coldframe exposure, or by watering with salt solutions. The much greater total water loss of the non-hardened plants was due to a large extent to the fact that they were larger than the hardened plants, though of the same age.

The fact that the rate of transpiration per unit of leaf area was less in hardened plants is significant. If the rate of diffusion of water from the cells into the intercellular spaces determines the rate of transpiration, then a lower rate of transpiration would be associated with a greater water-retaining power on the part of the plant cells. This water-retaining power would be exerted when the plant's cells are exposed to water loss by freezing in the same way as when exposed to loss by transpiration or by drying.

RATE OF DEHYDRATION IN HARDENED AND TENDER PLANTS.

Since it was found that hardened plants exhibited a greater water-retaining power than non-hardened plants upon freezing, it was thought that the difference might be measured by the rate of water loss in similar tissues exposed to drying.

Mr. V. R. Boswell[8] undertook a special investigation of the rate of dehydration of leaves from hardened and non-hardened plants during the winter and spring of 1921. The material used in his experiments was from the same lots upon which other results are reported in this paper.

Leaves of uniform condition and from corresponding parts of plants were gathered from cabbage and tomato plants subjected to various hardening treatments. Lots directly comparable were gathered and dried at the same time. The samples were placed in stoppered bottles, taken at once to the laboratory, weighed, and immediate-

TABLE 11.—RATE OF WATER LOSS BY DRYING AT 60°C. IN HARDENED AND TENDER LEAVES.
(In per cent of total moisture content)

Time in minutes	Tomato leaves		Cabbage leaves				
	Greenhouse wet-grown (tender)	Hardened in cold-frame	Greenhouse wet-grown (tender)	Hardened in cold-frame	Greenhouse plants		
					water (tender)	$NaNO_3$ (medium hardy)	NaCl (hardy)
15	34.77	26.46	21.53	8.92	23.82	19.70	12.13
30	68.11	57.91	42.71	17.43	46.71	38.68	24.29
45	83.99	75.34	54.20	36.83	62.74	54.80	34.68
60	94.27	87.85	75.32	47.56	74.81	66.27	43.13
75	97.94	95.57	79.08	55.23	84.67	76.42	51.99
90	99.34	99.02	93.32	68.23	91.59	83.20	59.36
105	99.59	99.52	93.12	74.58	96.32	89.27	66.79
120	99.62	99.61	99.66	86.01	98.73	93.94	73.34
135	99.64	99.63	98.11	94.88	99.68	97.57	78.87
150	.....	.....	99.80	94.73	99.93	99.37	84.48
165	.....	.....	.....	98.88	99.95	99.87	88.91

ly placed in an electric oven at a constant temperature of 60°C. The leaves were spread out on wire gauze placed on the shelves of the oven until they were beginning to become brittle, after which they were transferred to the weighing bottles in which the dehydration was completed. Each lot of leaves was removed from the oven at intervals of 15 minutes, cooled and weighed. From the loss in weight for each period of drying, was calculated the percent of total moisture removed per period. Table 11 compiled from some of Boswell's data, gives the percent of total moisture lost at the end of each 15-minutes interval in samples of hardened and tender plants.

The data presented in Table 11 bring out a striking difference in the rate of water-loss by drying in hardened and non-hardened leaves of cabbage, especially at the beginning of the period of drying. This difference is not very great in the two lots of leaves of tomato. In cabbage, leaves from plants hardened by exposure in the cold-frame, by watering with salt solutions and by partially withholding water, show a much smaller loss of water for each period than do leaves of tender, well-watered plants grown in the greenhouse. This difference in rate of drying indicates a relatively much greater water-retaining power in hardened plants. Whatever the differences in the two types of plant tissue are, the greater water-retaining power of the hardy tissue evidently does not depend entirely on the organization of living matter, but on the chemical and physical properties of the substances of which the tissues are composed.

Another point which may be seen from Boswell's dehydration experiments is that tomato leaves dry out much more rapidly than cabbage leaves. Even the hardened tomato leaves give up water faster than the leaves of non-hardened cabbage. In view of the fact that the tomato is not susceptible of hardening to the extent of surviving ice formation, it seems that we have here an indication of the fundamental difference between the two types of plants. The tomato lacks the potential ability to acquire or develop increased water-retaining power to any great degree, while the cabbage and similar plants have this potentiality to a considerable degree.

Figure 7 shows graphically the relative rate of water-loss by drying in the different types of tissue.

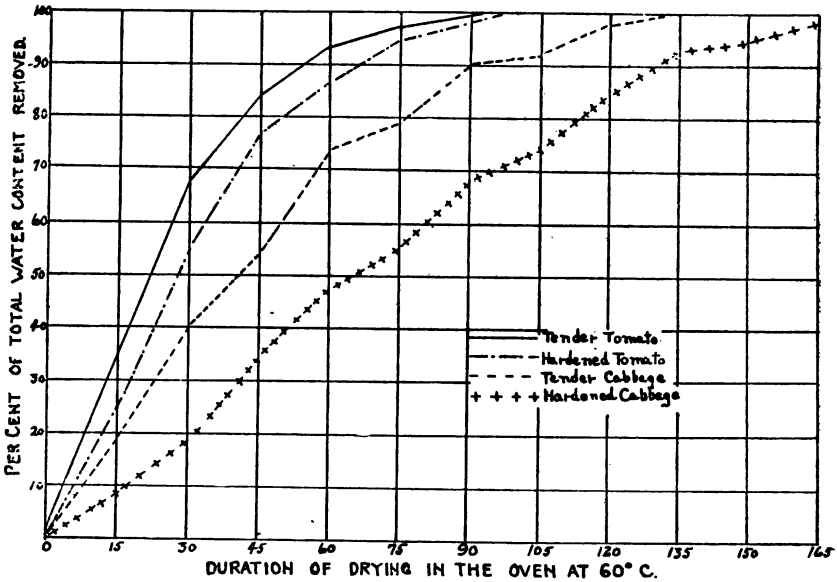

Fig. 7.—Rate of water loss from leaves of varying degrees of hardiness.

CHANGES IN CARBOHYDRATES ON HARDENING OF PLANTS.

Formation of sugar by low temperature.—Numerous investigators have noted increased amounts of sugars in plants exposed to low temperature. Mer[67] was probably the first to note the disappearance of starch and the accumulation of sugar in evergreen leaves in winter.

Lidforss[60] noted in a number of evergreen plants in Sweden that starch was converted to sugar in the fall and reconverted into starch in spring. He found that tender seedlings placed in a sugar solution for a short time were able to withstand several degrees of lower temperature without injury. Lidforss thought the hardiness of the evergreen leaves and the sugar-treated seedlings was due to increased concentration of the cell sap resulting from the accumulation of sugar, to which he attributed reduced transpiration and lower freezing point depression, as well as a protective effect of sugar on the precipitation of proteins of the cell. Gorke[35] found that he could prevent the precipitation of protein from expressed plant sap by adding sugar.

Miyake[70] examined the leaves of evergreen plants in various parts of Japan in winter finding those of many plants to be starch-free during the coldest part of winter. Another group had very little starch in the mesophyll during cold weather (when the mean temperature was near or below the freezing point.) Plants in Northern Japan were markedly lower in starch than in the warmer sections. Schulz[111] examined one hundred species of plants in Germany, finding most of them starch-free in winter, while a few contained a little starch mostly in the fibrovascular bundles and the surrounding cells.

Recently, Swedish investigators[2] have shown that the hardier varieties of wheat have a larger sugar content in fall and winter. They found that the percentage of dry matter and the amount of sugar in winter wheat varies considerably during the winter, fluctuating with the temperature, but during the period from November 12 to February 15, no starch could be found in the leaves. Gasner and Grimme[31] upon analyzing the first leaves of wheat, found that seedlings germinated at 5–6°C. had a greater sugar content than those germinated at 28°C. They also found a higher sugar content in leaves of hardy winter wheats than in spring wheats germinated at the same temperature.

Micheal-Durand[69] in extensive studies on the changes of carbohydrates in plants, found an enormous accumulation of sugars in leaves of certain evergreens in winter, while starch completely disappeared during the coldest weather. He explains this condition as follows:

(1) In winter assimilation is low, but respiration is depressed still more by the low temperature.
(2) Conditions in winter are unfavorable for translocation.
(3) Low temperature prevents the condensation of the simple sugars into higher carbohydrates.
(4) Breaking up (splitting) of polysaccharides.

Müller-Thurgau[75] and others, have measured the accumulation of simple sugars in potatoes at the expense of starch upon exposure to low temperature and the reconversion of the sugars into starch when higher temperatures are provided. This reversible chemical change seems to be generally associated with changing temperatures near the freezing point, probably due to shifting of chemical equilibrium by enzymatic activity.

Relation of sugar content to cold resistance.—Since the experiments of Lidforss[60] and Gorke[35] the extensive formation of

TABLE 12.—CHANGES IN CARBOHYDRATE CONTENT OF PLANTS SUBJECTED TO HARDENING TREATMENTS.

Series No.	Treatment	Date sampled	Per cent on dry weight basis: Reducing sugars	Total sugars	Starch	Total polysaccharides	Per cent on fresh weight basis: Reducing sugars	Total sugars	Starch	Total polysaccharides	% Dry matter
	Cabbage										
A1	Grown wet, in greenhouse (tender)	11/22/19			4.222	10.31			0.363	0.954	8.6
		3/12/20	1.10	2.58	3.69	7.66	0.088	0.212	0.302	0.628	8.2
		12/10/20	1.25	1.63			0.127	0.165			10.18
A2	Grown medium dry, in greenhouse	11/22/19				9.56				1.121	11.71
		3/12/20	3.50	4.00	2.82	5.52	0.396	0.451	0.319	0.631	11.3
A3	Grown dry in greenhouse (hardy)	12/8/19			3.125	9.26			0.312	0.926	10.00
		3/12/20		4.67	2.19	6.24		0.597	0.281	0.798	12.80
		4/5/20				8.94				1.019	11.40
		12/10/20	5.50	6.62			0.651	0.784			11.85
A4	Water partially withheld for 2 weeks (in greenhouse)	3/12/20	2.25	3.42	2.50	7.82	0.242	0.368	0.269	0.841	10.76
E4	Greenhouse plants, not hardened	3/22/20	1.78	3.58	3.13	7.13	0.187	0.376	0.329	0.748	10.52
		4/5/20				8.25				0.774	9.38
E3	Hardened in coldframe 1 week	3/20/19	8.74			1.422	0.986				11.28
		11/22/19	8.870	10.670	2.037	8.00	1.040	1.250	0.239	0.938	11.71
		3/22/20		6.00	3.44	7.26		0.738	0.424	0.893	12.31
E2	Hardened in coldframe 2 weeks	3/20/19		11.44				1.514			13.24
		3/22/20	8.26	10.75	0.72	8.26	1.067	1.388	0.093	1.067	12.91
		12/10/20	4.87	8.59			0.614	1.084			12.62
E1	Hardened in coldframe 3 weeks	11/22/19	3.316	4.75	2.319	10.31	0.416	0.596	0.291	1.295	12.55
		12/8/19		4.66	1.444	9.31		0.515	0.159	1.028	11.05
		3/22/20	5.65	11.00	trace	6.59	0.751	1.461	0.000	0.874	13.27
		4/5/20	6.44	9.54		6.25	0.794	1.176		0.771	12.33
	Lettuce										
A1	Greenhouse plants grown wet (tender)	10/31/19	1.195	5.67	8.06	8.25	0.066	0.317	0.445	0.454	5.52
A3	Greenhouse plants grown dry (hardy)	10/31/19	2.095	8.10	7.25	9.36	0.138	0.532	0.476	0.614	6.56
B6	Greenhouse plants grown in rich soil (very tender)	10/31/19	1.350	6.17	8.20	8.87	0.072	0.328	0.436	0.472	5.32

TABLE 12.—(Continued)

Series No.	Treatment	Date sampled	Per cent on dry weight basis: Reducing sugars	Total sugars	Starch	Total polysaccharides	Per cent on fresh weight basis: Reducing sugars	Total sugars	Starch	Total polysaccharides	% Dry matter
	Cabbage										
B2	Greenhouse plants grown in poor sandy soil (med. hardy)	10/31/19	1.870	8.17	5.82	7.145	0.096	0.420	0.299	0.367	5.13
X1	Greenhouse plants (very tender)										
	old plants	11/29/19	0.709	3.008		6.63	0.028	0.121		0.264	3.98
	young plants	3/24/20	6.603	9.50	3.56	7.72	0.521	0.746	0.280	0.605	7.85
	older plants	12/10/20	1.66	7.92			0.102	0.485			6.115
X2	Coldframe plants										
	old plants	11/29/19	2.880	7.113	2.25	7.50	0.202	0.499	0.158	0.527	7.02
	young plants	3/24/20	8.00	20.00	3.37	8.19	0.840	2.100	0.354	0.860	10.50
	older plants	12/10/20	5.35	13.75			0.340	0.874			6.38
	Head Lettuce										
	Hardened in coldframe										
	young plants	3/24/20	4.94	22.32		5.59	0.556	2.487		0.622	11.13
	old plants	12/10/20	3.94	16.00			0.392	1.594			9.97
	Cauliflower										
E2	Greenhouse plants not hardened	3/24/20	5.00	8.25		10.42	0.593	0.982		1.240	11.9
E1	Hardened in coldframe	3/24/20	7.31	10.42	1.69	8.69	0.981	1.374	0.226	1.174	13.4
	Tomato										
A1	Wet-grown greenhouse	5/3/19				3.37				0.419	12.41
	plants, tender	9/21/19	0.788	3.42	5.87	8.32	0.065	0.285	0.488	0.691	8.29
A3	Dry grown greenhouse	5/3/19		1.80		7.54		0.320		1.346	17.78
	plants, hardy	9/21/19	1.236	2.85	14.18	16.61	0.135	0.313	1.56	1.711	11.00
E5	Greenhouse plants not hardened	5/3/19	2.295	3.457	3.56	4.598	0.324	0.488	0.502	0.662	14.1
E3	Hardened in coldframe, 10 days	5/3/19	1.257	2.295	12.0	27.45	0.184	0.335	1.755	4.010	14.6
E1	Hardened in coldframe, 20 days	5/3/19	2.08	2.83		23.28	0.288	0.406		3.416	13.65

TABLE 12.—CHANGES IN CARBOHYDRATE CONTENT OF PLANTS SUBJECTED TO HARDENING TREATMENTS.

Series No.	Treatment	Date sampled	Per cent on dry weight basis: Reducing sugars	Total sugars	Starch	Total polysaccharides	Per cent on fresh weight basis: Reducing sugars	Total sugars	Starch	Total polysaccharides	% Dry matter
	Cabbage										
A1	Grown wet, in greenhouse (tender)	11/22/19			4.222	10.31			0.363	0.954	8.6
		3/12/20	1.10	2.58	3.69	7.66	0.088	0.212	0.302	0.628	8.2
		12/10/20	1.25	1.63			0.127	0.165			10.18
A2	Grown medium dry, in greenhouse	11/22/19				9.56				1.121	11.71
		3/12/20	3.50	4.00	2.82	5.52	0.396	0.451	0.319	0.631	11.3
A3	Grown dry in greenhouse (hardy)	12/8/19			3.125	9.26			0.312	0.926	10.00
		3/12/20		4.67	2.19	6.24		0.597	0.281	0.798	12.80
		4/5/20				8.94				1.019	11.40
		12/10/20	5.50	6.62			0.651	0.784			11.85
A4	Water partially withheld for 2 weeks (in greenhouse)	3/12/20	2.25	3.42	2.50	7.82	0.242	0.368	0.269	0.841	10.76
E4	Greenhouse plants, not hardened	3/22/20	1.78	3.58	3.13	7.13	0.187	0.376	0.329	0.748	10.52
		4/5/20				8.25				0.774	9.38
E3	Hardened in coldframe 1 week	3/20/19	8.74			1.422	0.986				11.28
		11/22/19	8.870	10.670	2.037	8.00	1.040	1.250	0.239	0.938	11.71
		3/22/20		6.00	3.44	7.26		0.738	0.424	0.893	12.31
E2	Hardened in coldframe 2 weeks	3/20/19		11.44				1.514			13.24
		3/22/20	8.26	10.75	0.72	8.26	1.067	1.388	0.093	1.067	12.91
		12/10/20	4.87	8.59			0.614	1.084			12.62
E1	Hardened in coldframe 3 weeks	11/22/19	3.316	4.75	2.319	10.31	0.416	0.596	0.291	1.295	12.55
		12/8/19		4.66	1.444	9.31		0.515	0.159	1.028	11.05
		3/22/20	5.65	11.00	trace	6.59	0.751	1.461	0.000	0.874	13.27
		4/5/20	6.44	9.54		6.25	0.794	1.176		0.771	12.33
	Lettuce										
A1	Greenhouse plants grown wet (tender)	10/31/19	1.195	5.67	8.06	8.25	0.066	0.317	0.445	0.454	5.52
A3	Greenhouse plants grown dry (hardy)	10/31/19	2.095	8.10	7.25	9.36	0.138	0.532	0.476	0.614	6.56
B6	Greenhouse plants grown in rich soil (very tender)	10/31/19	1.350	6.17	8.20	8.87	0.072	0.328	0.436	0.472	5.32

TABLE 12.—(Continued)

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	Cabbage										
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X1	Greenhouse plants (very tender)										
	old plants	11/29/19	0.709	3.008		6.63	0.028	0.121		0.264	3.98
	young plants	3/24/20	6.603	9.50	3.56	7.72	0.521	0.746	0.280	0.605	7.85
	older plants	12/10/20	1.66	7.92			0.102	0.485			6.115
X2	Coldframe plants										
	old plants	11/29/19	2.880	7.113	2.25	7.50	0.202	0.499	0.158	0.527	7.02
	young plants	3/24/20	8.00	20.00	3.37	8.19	0.840	2.100	0.354	0.860	10.50
	older plants	12/10/20	5.35	13.75			0.340	0.874			6.38
	Head Lettuce										
	Hardened in coldframe										
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	old plants	12/10/20	3.94	16.00			0.392	1.594			9.97
	Cauliflower										
E2	Greenhouse plants not hardened	3/24/20	5.00	8.25		10.42	0.593	0.982		1.240	11.9
E1	Hardened in coldframe	3/24/20	7.31	10.42	1.69	8.69	0.981	1.374	0.226	1.174	13.4
	Tomato										
A1	Wet-grown greenhouse plants, tender	5/3/19				3.37				0.419	12.41
		9/21/19	0.788	3.42	5.87	8.32	0.065	0.285	0.488	0.691	8.29
A3	Dry grown greenhouse plants, hardy	5/3/19		1.80		7.54		0.320		1.346	17.78
		9/21/19	1.236	2.85	14.18	16.61	0.135	0.313	1.56	1.711	11.00
E5	Greenhouse plants not hardened	5/3/19	2.295	3.457	3.56	4.598	0.324	0.488	0.502	0.662	14.1
E3	Hardened in coldframe, 10 days	5/3/19	1.257	2.295	12.0	27.45	0.184	0.335	1.755	4.010	14.6
E1	Hardened in coldframe, 20 days	5/3/19	2.08	2.83		23.28	0.288	0.406		3.416	13.85

sugars in leaves of plants exposed to cold has generally been considered to be related to their cold-resistance. However, Harvey[42] concluded that carbohydrate changes were not important in the hardening process with cabbage plants, since he found that cabbage plants could be hardened to some extent at least, by keeping them several days in the dark in a low temperature chamber, during which time there was little change in the carbohydrate equilibrium. However, it has been shown by several investigators and notably by Lewis and Tuttle[59] that simple sugars form a large part of the osmotically active cell contents.

From the beginning of these experiments, samples were collected for carbohydrate analyses from some of the series of plants in each of the hardening treatments. The results of some of these determinations are given in Table 12.

Methods of analysis.—The sugar analyses were made according to the modified Munsen and Walker method, as described by Hooker,[46] the results being expressed as dextrose.

One gram of the air dry, ground plant material was weighed, transferred to filter paper and washed thoroughly five times with distilled water. The insoluble residue was used for the starch determination. The filtrate, amounting to about 150 cc., was taken for determination of soluble sugars. After clearing with basic lead acetate the extract was made up to 250 cc. and filtered. Two hundred cc. of the filtrate was pipetted into a volumetric flask, excess lead precipitated with solid sodium carbonate, made up to 250 cc. and filtered. An aliquot of the filtrate (Solution A) was used for the determination of reducing sugars, while another portion was used for determination of the total sugars.

Five cc. of concentrated HCl was added to 75 cc. of Solution A and hydrolized at 70°C. for exactly ten minutes (Solution B). After cooling, this solution was neutralized with sodium hydroxide made up to 100 cc. and used for the determination of total sugars as dextrose.

The sugar-free residue of the original sample was used for the starch determination. It was washed into a beaker, boiled five minutes to convert the starch into a paste and after cooling 3 cc. of Taka-diastase solution were added. The beaker was then placed in the oven at 40°C. for 24 hours, the starch being broken down to maltose and dextrin. The liquid containing these sugars was then filtered off, adding the washings to the filtrate, which was hydrolized with acid for 2½ hours under a reflux condenser to break down further the products of digestion to dextrose. After cooling, the solution was neutralized with sodium hydroxide, cleared and prepared for analysis as previously described. A blank with the same amount of Taka-diastase solution was run with each series of starch determination.

Total polysaccharides were determined on a sample of the dry plant material washed free of soluble sugars with cold water. The filter paper was punctured and the residue washed into a 700 cc. flask. Eight cc. concentrated HCl. and enough water was added to bring the total volume to 150 cc. After boiling two and one half hours under reflux condenser, the contents of flask were cooled, transferred to a beaker and made neutral to litmus with sodium hydroxide. The solution was then prepared for analysis as previously described for Solution A.

Discussion.—Table 12 presents evidence that: (1) The content of both reducing and total sugars increases in hardened plants. This increase seems to be greater in plants hardened by exposure to low temperature in the coldframe than in plants hardened by other methods. The increase in sugar is greater in hardened cabbage and lettuce than in the tomato, though there is no direct evidence that the absolute quantity of sugars present in the plant is directly related to its cold-resistance. Thus some of the tender lettuce samples have more sugar than certain samples of hardy cabbage. Young lettuce plants contained much more sugar than plants approaching maturity and this may have something to do with the greater cold-resistance Chandler[20] found in the younger leaves of lettuce, whereas in most plants the young leaves were somewhat more tender to cold.

(2) In lettuce, cauliflower and cabbage the amount of total polysaccharides is usually somewhat less in hardened than in non-hardened plants, which decrease may be attributed to the reduction in the amount of starch. In the tomato, on the other hand, the total polysaccharides show a large increase, apparently due mostly to the deposition of starch in large quantities in both stems and leaves of plants exposed to any of the hardening treatments. Kraus and Kraybill[54] found a similar increase of starch in tomato plants in a stunted condition. Hartwell[48] found a large accumulation of starch in plants, especially the potato, when the growth was checked by any limiting factor.

Here is an interesting distinction between the chemical changes in a group of plants susceptible of considerable hardening to cold and a plant not susceptible of much hardening. In the group of plants possessing potential hardiness, exemplified by the cabbage, any hardening treatment causes a considerable increase in sugars and a decrease in starch, while the total polysaccharide figure remains nearly constant (on the fresh weight basis) because, as will be shown later, of an increase in pentosans. On the other hand in the tomato, lacking potential hardiness, the hardening treatments caused only a slight increase in the sugars and an enormous increase in polysaccharides due mostly to an increased starch content.

An increased sugar content in the hardened plants would increase the osmotic concentration of the cell sap, depress the freezing point and perhaps serve to hold a somewhat larger amount of water in the unfrozen state when the plant is exposed to low temperatures. However, the importance of the increased content of sim-

ple sugars in cold resistance remains undetermined, nor is it known to what extent sugars may be responsible for the greater water-retaining capacity of hardened tissues. It appears probable that an increased sugar content in hardened plants is more likely one of the manifestations of the condition of being hardy than a direct cause of cold resistance.

NATURE OF WATER-RETAINING POWER IN PLANTS.

It has been shown by experiments with the dilatometer that: (1) the amount of water frozen in hardened cabbage plants is considerably less than in tender plants, (2) the increase in the amount of water frozen as the temperature is lowered becomes less and less, probably approaching zero, (3) the amount of water freezing at a given temperature (–5°C.) decreases as the degree of hardening increases. It has also been shown that hardened plants have a lower transpiration rate and that hardened tissues dry out more slowly than tender tissues. The greater water-retaining power of the cells of hardened plants must therefore be accepted as a fact. What factors are responsible for the development of this increased water-retaining power?

Several investigators have attached great significance to the osmotic concentration of the sap as determined by the depression of its freezing point. Some data are also presented in this paper (Table 2) showing that in hardened plants this depression is greater than in non-hardened plants. However, concentration of the sap, even if entirely due to substances having a low eutectic point, would not be sufficient to account for the amount of water found to remain unfrozen in hardened plants (Table 3). Moreover, some of the sap solutes have a high eutectic point, for Harvey[42] found numerous large crystals of calcium malophosphate in frozen spots on leaves exposed to temperatures not low enough to kill the whole plant. Some investigators have stated that the increased sugar content of plants in winter was at least to some extent responsible for their cold-resistance because of the increased concentration thereby imparted to the cell sap. The highest percentage of total sugar found in cabbage in these experiments (Table 12, 1.461 percent in Series E1, gathered March 22, 1920,) is equal to only 1.68 percent sugar solution in the plant sap. Considering half of this sugar to be glucose and half sucrose, we have a sugar solution equivalent to less than 0.075 molecular. This would not be sufficient to affect materially the amount of water frozen in the plant tissue at a point several degrees

below 0°C., one gram-molecular weight of a non-electrolyte lowering the freezing point 1.86°C.

It has been further pointed out (p. 35) that the greater depression of the freezing point in hardy tissues is associated with certain changes in the plant cell upon hardening, the apparent increase in sap concentration being simply an accompaniment, rather than a cause of increased hardiness. It therefore is necessary to introduce some other factor to explain the difference in amount of water freezing in hardy and in tender tissues. It has been indicated that the force of imbibition may be a powerful factor in withholding water from freezing. This force varies inversely to the water content, but probably increases more rapidly than the rate of decrease in water content, as indicated by the slow rate of drying leaves and of colloidal materials after they have been dried out to a certain extent. It is a pretty well recognized fact that tissues with lower water content are more resistant to killing by cold.

Plant protoplasm is not a compound of definite chemical composition or even constant physical condition, but a colloidal mixture of the emulsoid type varying in consistency from a hydrosol to that of a hydrogel and containing different substances which may be present in greater or less amounts at different times and in different organs. According to Seifriz,[113] the change from one state to another is dependent upon, or coincident with, changes in physiological activity. Thus, in the eggs of *Fucus,* he found a progressive increase in viscosity with decreasing physiological activity. Strausbaugh,[117] as a result of recent investigations on the plum in Minnesota, suggests that the prolonged dormancy and water-retaining power which he found in hardy varieties is due to a change in colloidal properties creating an increased power of imbibition. The work of these investigators is significant, since hardy plants are usually at a low state of physiological activity at the time of their greatest cold resistance.

Water of imbibition may be held by molecular capillarity or in the absorbed condition by the hydrophilous colloids of the plant cells. Such water is not readily available for freezing, in other words, the force with which it is held must be overcome by a considerable force of crystallization before it can be drawn from the cell and frozen. Mc Cool and Millar[80] have suggested the classification of plant moisture as ''free' or easily freezable and ''unfree'' or not easily freezable, somewhat as Bouyouces has classified soil water. Such a classification necessitates setting an arbitrary temperature of freezing, the

relative amounts of free and unfree water varying with the temperature at which freezing takes place. Yet it is convenient for our present purpose to refer to free and unfree water in the sense that the latter, for one reason or another, remains unfrozen at a given temperature.

It seems from the work of Bouyoucos[10] and of McCool and Millar[80] that the unfree water is held to a very large extent in the adsorbed condition by protoplasmic colloids. The water-retaining power of colloids and the quantity of certain colloidal materials in the cell are thereby suggested as an explanation of increased water-retaining power and cold-resistance in plants.

Relation of pentosan content to cellular water-retaining power.—Spoehr's work[116] on cacti suggests that pentosans may be the specific substances which increase the water-retaining power in hardened plants. He found that the pentosan content of *Opuntia* increased considerably under xerophytic conditions and suggested that the large water-retaining power of the pentosans is largely responsible for their well-known ability to survive under such circumstances. The work of Livingston[138] and others has shown that the osmotic pressure in cacti and other desert plants is no greater than in many mesophytes, hence this factor probably plays only a small part in the water-holding power of most xerophytic plants.

Spoehr found by analysis of desert plants that in cells undergoing water depletion, other polysaccharides were changed to pentosans, of which the plant mucilages are largely composed. Thus, "undue loss of water caused a change in the cell whereby the amount of water it may hold is greatly increased." Mac Dougal[86] considers a change of this sort to be the basis of xerophytism. Water of imbibition was found by Spoehr to be closely related to the presence of the pentose polysaccharides. Pentosan formation increased decidedly with low and decreased with high water content. From April till June, while the weather was very dry, pentosans made up from 9 to 12 percent of the dry weight. During the rainy weather of July, the pentosan content fell to 4.39 percent of the dry weight, increasing to 12.5 percent again in the dry cool weather of the fall and falling to 4.37 percent when the winter rains set in.

Not all cacti possess a large pentosan content. Spoehr gives analysis of two species of *Opuntia* growing at Tucson, as follows:

		Fresh weight basis			
	% water	% total sugars	% total polysach.	% total pentose	% pentosan
O. Versicolor	82.15	1.97	1.50	0.36	0.230
O. Phaeacantha	78.70	3.53	3.22	1.64	1.550

In view of this difference in composition, it may be significant that *O. Phaeacantha* is listed in Bailey's Encyclopedia of Horticulture as a hardy variety and is reported by Shreve to grow in the mountains about Tucson to an altitude of 7500 feet.

A striking property of the pentosans is their power of swelling and taking up an enormous amount of water, which the hexose polysaccharides do not do to nearly so marked a degree. The occurrence of pectins in the middle lamella of the cell walls is well known. Spoehr believes that they are also distributed through the protoplasm and are used for a variety of purposes. The plant nucleo-protein has been found by Levine and Jacobs[186] to contain the pentose group as part of the nucleic acid radical. Tollens[187] showed that pentosans were widely distributed in plants and were limited to no special tissue, but abundant in roots, stems, leaves and seeds. He found further that pentosans showed all possible variations as to solubility in water. Swartz[119] obtained a water-soluble pentosan from *Dulce.* In the crude form, this was very hygroscopic, but this property was lost after several purifications. She found that the hemi-celluloses of ten species of marine algae were chiefly pentosans and galactans and concluded that pentosans and hexosans very commonly occur together, not only intimately associated, but chemically combined. Mac Dougal[81] goes so far as to state that the "plant protoplasm consists of a comparatively inert base of pentosans—in colloidal combination with proteins, amino acids, lipins, and salts."

As to the origin of pentosans, Spoehr[116] shows that pentoses can be formed from the hexosans as the first product of oxidation. This view is corroborated by the work of Ravena and Cereser,[102] who found no marked variation in pentosan content during the period of photosynthetic activity, but when the carbohydrate food consisted entirely of dextrose, the amount of pentosans increased greatly, especially in light. The probability of pentosan formation from the hexosans is indicated also by the increased pentosan content in the presence of high total sugar and diminishing starch, as shown later in this paper.

Davis, Daish and Sawyer[24] found no diurnal variation in the pentosan content of plants. However, they found that the amount of pentosan in the leaf of the Mangold (*Beta vulgaris*) increased from August to October.

Hornby[48] found that the pectin content varied in different parts of the same plant. More pectin was found in the epidermal tissue than in the cortex. Exposure to light, and mechanical injury to

tissues, were found to result in increased pectin content in the exposed or injured part. Hornby suggested that pectin might have a protective effect on plants, especially against insect attacks.

Hooker[47] has shown that the hardier parts of apple shoots, the bases, have a greater water-retaining power than the tips, which are less cold-resistant. He placed portions of the air-dried ground material in desiccators containing sulfuric acid, the concentration of which ranged from 100 to 36.69 percent. The air-dry material lost moisture in the desiccators containing the higher concentration of acid and this loss was greater in the tender material. But over the lowest concentration of acid used, water was taken up, the gain in weight being greater in the hardy material. This experiment indicates that hardy apple twigs contain a larger amount of some hygroscopic material. Hooker attributed the greater water-retaining power of the hardy tissue to the larger percentage of total pentosan found therein.

Pentosan content in the hardening process in vegetable plants.—In this work, a study was made of the pentosan content in an effort to throw light on the nature of the increased water-retaining power of hardened plants. For the pentosan determinations, samples were taken from plants grown under the various hardening treatments previously described. Also a series of analysis were made on plant material gathered from the field at intervals during the fall of 1920.

Method of Pentosan Analysis.—The method of analysis was that employed by Spoehr.[116] A two gram sample of the oven-dry material was hydrolized by boiling for three hours with eight cc. concentrated HCl in 150 cc. water. After cooling, the entire contents of the flask containing the products of hydrolysis were transferred to a 400 cc. baker, neutralized with NaOH, a uniform amount of a suspension of yeast was added and the beakers placed in an oven at 35–40°C. over night. The hexose sugars were fermented off, leaving the non-fermentable pentose sugars in the solution. After fermentation the material was filtered and washed, the filtrate containing the pentose sugars was boiled ten minutes to drive off the alcohol, then prepared for analysis in the same way as described for sugar determinations. The result obtained was calculated from Munson and Walker's tables, multiplying the glucose value by 0.85, since Spoehr found that the reducing value of the pentose sugars held that relation to glucose. The results on total pentosan content are given in Table 13.

TABLE 13.—TOTAL PENTOSAN CONTENT OF PLANTS FROM VARIOUS HARDENING TREATMENTS.

Serial No.	Treatment	Cabbage Date sampled	Cabbage on fresh wt. basis	Cabbage on dry wt. basis	Tomato Date sampled	Tomato on fresh wt. basis	Tomato on dry wt. basis	Leaf Lettuce Date sampled	Leaf Lettuce on fresh wt. basis	Leaf Lettuce on dry wt. basis
A1	Grown in greenhouse. optimum moisture, (tender)	12/18/19	.289%	4.06%	5/3/20	0.693%	7.10%	10/5/20	0.106%	2.12%
		3/12/20	.243	2.97	4/30/21	0.382	3.93			
		3/22/20	.352	4.59	12/12/19	0.341	2.65			
		3/22/20	.323	4.42						
		3/16/21		3.61						
	(light shade)	3/19/21	.160	2.55						
A2	Medium moisture	3/12/20	.320	2.82	5/3/20	0.795	5.39			
		12/10/20	.429	4.21	4/30/21	0.401	3.67			
		3/19/21	.527	5.26	9/21/19	0.455	5.10			
					10/16/19	0.798	6.11			
A3	Minimum moisture (hardy)	11/20/19	.541	4.41	5/3/20	0.720	4.56	10/5/20	0.402	4.31
		12/18/20	.583	5.83	4/30/21	0.575	4.68			
		3/12/20	.423	3.30	12/12/19	0.596	2.47			
		3/22/20	.540	5.15	12/12/19	0.548	4.68			
		3/22/20	.623	8.19	10/16/19	0.780	6.11			
		4/5/20	.563	4.94						
		12/10/20	.631	5.32						
		3/16/21	.525	5.00						
		3/19/21	.519	4.94						
A4	Water withheld 2 weeks	3/12/20	.412	3.83	5/3/20	0.598	4.54	10/5/20	0.564	6.489
		3/19/21	.527	5.37	9/21/19	0.464	4.41			
E4	Greenhouse plants, not hardened	3/22/20	.207	1.97	5/3/20	0.384	3.45	11/29/19	0.131	3.29
		4/5/20	.195	2.09				3/24/20	0.126	1.61
		3/29/21	.290	4.25						
E3	Hardened in coldframe, 1 week	3/19/21	.442	4.12	4/30/21	0.556	4.62			
		3/22/20	.413	3.35						
E2	Hardened in coldframe 2 weeks	12/8/19	.588	5.31	5/3/20	0.682	5.31	3/24/20	0.230	2.19
		3/22/20	.530	4.10	4/30/21	0.575	4.68	3/24/20	0.568	5.10
		12/10/20	.662	5.24				(head lettuce)		
E1	Hardened in coldframe 3 weeks	3/22/20	.810	6.10	5/3/20	0.362	2.93			
		3/19/21	.522	5.20	4/30/21	0.581	4.09			
E0	Hardened in coldframe							11/29/19	0.295	4.20
	4 weeks	4/5/20	.654	5.301	4/30/21	0.562	4.41	10/31/19	0.369	4.20
E00	Hardened in coldframe									
	5 weeks	3/16/21	.776	5.84						

Plants not hardened by any special treatment are low in total pentosans and hardened plants have a much larger amount, in some cases in cabbage an increase of about 200 percent. Plants given intermediate hardening treatments have a medium amount of pentosans. The increased pentosan content of the hardened plants is most striking if we consider the results on the fresh weight basis. This probably is the most suitable criterion to use in a study of the reactions which concern the living plant, especially since Parker has shown that the force with which water is held by finely divided materials depends largely on the moisture content.

It may seem that the absolute amounts of pentosans, even in the hardened plants, are too small to influence very markedly the force with which the cells may retain water under conditions of stress. However, it should be borne in mind that in nature the pentose molecule probably exists in combination with four molecules of galactose or other hexose sugar. Hence the amount of pentosans in the plant is much greater than the analyses indicate.

Pentosan content of garden plants.—Samples of leaves were gathered at intervals during the fall from cabbage, kale and celery plants growing in the open field. The seed had been sowed in July and the plants made considerable growth before the first light frost came on October 1. The month of October was mild, and the plants remained alive until heavy freezes the last of November. Exposed to steadily declining seasonal temperatures, these plants may be considered to have undergone a kind of hardening treatment, for they were able to withstand light frost in October and heavy frost the early part of November. The results of the total pentosan determinations are given in Table 14.

TABLE 14.—TOTAL PENTOSAN CONTENT OF GARDEN PLANTS IN AUTUMN.

Date sample collected	Kale		Cabbage		Celery	
	% of fresh wt.	% of dry wt.	% fresh wt.	% dry wt.	%of fresh wt.	% of dry wt.
Sept. 15			0.289	4.06		
Oct. 7	0.511	3.93	0.580	4.36	0.567	4.42
Oct. 20	0.528	4.89	0.545	4.73	0.801	4.26
Nov. 3	0.537	3.93	0.621	4.36	0.793	4.44
Nov. 10	0.722	4.95	0.782	5.31	1.029	5.58
Nov. 18	1.064	6.48				

Table 14 shows that the total pentosan content of these plants becomes high when they are exposed to cool weather during the late

fall. The pentosan content on the fresh weight basis increases fairly regularly up to date of last sampling.

Pentosan content in plants watered with salt solutions.—An experiment wherein the hardiness of cabbage plants was considerably increased by watering them with M/10 salt solutions has been described. Plants hardened in this way were shown to have greater water-retaining power than unhardened plants. Samples from the salt treatment plots were analyzed for total pentosan content. The results are given in Table 15.

TABLE 15.—PENTOSAN CONTENT IN CABBAGE PLANTS HARDENED BY SALT SOLUTIONS.

Treatment of plants	Percent total pentosans	
	On fresh weight basis	on dry wt. basis
Compost soil, tap water	0.290	4.25
Compost soil, $NaNO_3$	0.471	5.05
Compost soil, KCl	0.451	4.24
Compost soil, NaCl	0.483	5.05
Sand, tap water	0.220	3.45
Sand, NaCl	0.288	4.25

The total pentosan content of the plants whose growth was checked by the application of the salt solutions and which were hardier to cold, show somewhat greater amounts of total pentosans on the dry weight basis and a considerable increase on the fresh weight basis, as compared to plants making a normal growth with tap water.

It appears from Table 15, that the pentosan content of the plants grown in sand is considerably lower than for plants grown in compost soil and receiving corresponding treatments. The plants grown in sand and receiving tap water were somewhat tenderer to cold than those grown in compost and likewise given tap water. Plants grown in sand and watered with M/10 NaCl show only a slight increase in pentosan content, as compared to plants grown in compost soil, likewise watered with M/10 NaCl. Here again, pentosan content shows a close correlation with the hardiness of the plants, as determined by freezing experiments.

Rate of increase in pentosan content.—The three groups of experiments just described having indicated a larger amount of pentosans in plants hardened in different ways, it was deemed desirable to determine their rate of development during the hardening process. Lots of potted cabbage plants were removed from the warm green-

house at intervals during March, and placed in an open coldframe. On March 19, samples were taken for analysis from all the lots which had been exposed to the hardening process for periods ranging from 3 to 20 days, as well as from some of the original lot which had been kept in the greenhouse under favorable growing conditions. The total pentosan content of the plants hardened for varying lengths of time is given in Table 16.

TABLE 16.—RATE OF INCREASE OF THE TOTAL PENTOSAN CONTENT IN CABBAGE PLANTS.

Treatment	Percent pentosan	
	On fresh weight basis	On dry weight basis
Greenhouse plants, not hardened	0.260	2.97
Hardened in frame 3 days	0.374	3.56
Hardened in frame 5 days	0.442	3.86
Hardened in frame 10 days	0.750	5.00
Hardened in frame 20 days	0.776	5.84

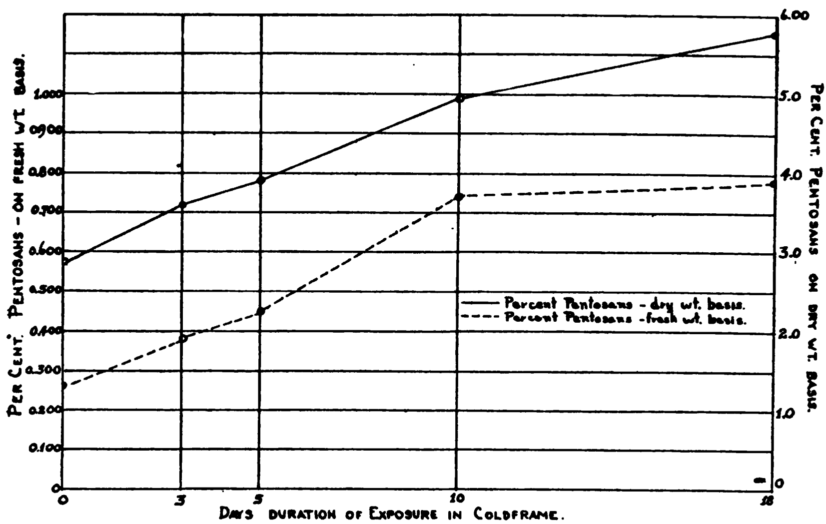

Fig. 8.—Rate of increase in total pentosan content of cabbage leaves during the hardening process.

The results of Table 16 are shown graphically in Figure 8. It appears that the increase in pentosan content proceeds quite rapidly and at a fairly uniform rate for ten days. After the first ten days of exposure in the coldframe the pentosan content increased only

slightly in this experiment. Other experiments have shown that the cabbage plant acquires nearly its maximum degree of hardening in this time. The dry matter content likewise increases rapidly the first few days of the hardening process, and more slowly thereafter.

In the dilatometer experiments, it was found that the amount of water frozen at –5°C. decreased with the duration of the hardening treatment in approximately the same order as the pentosan content is shown to have increased here. This seems to indicate a close relationship of pentosan content to water-retaining power and to cold resistance. The plants used in the dilatometer experiments were of the same lots as those from which the pentosan analyses were made.

TABLE 17.—RELATION OF HOT-WATER-SOLUBLE PECTINS TO TOTAL PENTOSAN CONTENT IN THE HARDENING PROCESS.

Treatment	Date sample taken	Percent pentosan on fresh weight basis		
		Total	Hot-water soluble	Insoluble (by difference)
Cabbage				
Wet-grown greenhouse plants	3/12/20	0.215	0.075	0.140
Dry-grown greenhouse plants	3/12/20	0.423	0.292	0.131
Greenhouse plants not hardened	3/22/20	0.207	0.091	0.116
Hardened in cold-frame 2 weeks	3/22/20	0.530	0.408	0.124
Hardened in cold-frame 3 weeks	3/16/21	0.776	0.550	0.226
Tomato				
Wet-grown in greenhouse	5/3/20	0.693	0.070	0.623
Dry-grown in greenhouse	5/3/20	0.720	0.071	0.649
Greenhouse plants not hardened	5/3/20	0.384	0.051	0.333
Hardened in cold-frame 2 weeks	5/3/20	0.682	0.071	0.611
Sweet Potato				
Garden plant	10/7/20	0.477	0.127	0.350
Kale				
Garden plant	10/7/20	0.511	0.223	0.288
Garden plant	11/18/20	1.064	0.418	0.646
Celery				
Garden plant	10/7/20	0.567	0.236	0.331
Garden plant	11/10/20	0.793	0.423	0.370

Relation of hot-water-soluble pentosans to the hardening process.—In jelly-making a hot water extract of fruits is used. According to Goldthwaite[33] a cold water extract of our common fruits contains little or no pectin. The total pentosan determinations given in the four preceding tables indicate the larger content of pentosans in hardened plants, but in the total pentosans is included probably a more or less considerable amount of the insoluble hemi-celluloses of the cell wall, which might not be expected to function to any great extent as water-retaining material, though undoubtedly a part of the power of imbibition of the plant cell is due to its walls. The experience of jelly makers indicates that the hot water extract of fruit contained the most of the jelly-forming pectins. It was thought, therefore, that a hot water extract of the plant material would yield approximately that fraction of the total pentosan which exists in the protoplasm and might function as the significant water-retaining material.

Accordingly, analyses were made from some of the samples, varying the procedure from that described for the total pentosan determinations as follows: The weighed sample of dry material was transferred to a beaker with 150 cc. of distilled water. The slight acidity was neutralized by adding a bit of sodium carbonate, then the material was boiled for five minutes, and filtered hot through a Gooch crucible. This yielded a clear cherry-colored filtrate, containing all the hot-water-soluble pentosans, sugars, and other soluble carbohydrates. Hydrolysis, fermentation, clearing and analysis were carried out with this filtrate in the same way as previously described for the whole sample in the total pentosan determinations. The results are given in Table 17.

In cabbage plants exposed to hardening treatment, the water-soluble pentosans increase considerably while the insoluble (hemi-cellulose) fraction is nearly constant, regardless of the degree of hardiness. In hardened cabbage plants the amount of soluble pentosans is relatively large, in fact the increase in the total pentosan content is very largely due to the increase in the water-soluble fraction.

In tomatoes, on the other hand, the hot water soluble fraction is very small and does not increase much in plants subjected to hardening treatments. The relatively large amount of total pentosans in the tomato, therefore, is largely insoluble, probably existing mostly as hemi-cellulose or in the middle lamella. The sweet potato resembles the tomato, in that it has a relatively large total

pentosan content, but only a small soluble fraction. The sweet potato, like the tomato, is very tender to frost and is not susceptible of much increase in cold-resistance upon exposure to usual conditions of hardening.

The water-soluble fraction of the total pentosan content in garden plants of kale and celery is shown to increase considerably as they become hardier in the fall.

These differences in the soluble pentosan content may give us an important clue to the reason for the previously shown difference in cold-resistance, susceptibility to hardening and water-retaining power in the two groups of plants represented respectively by the cabbage and the tomato.

Factors influencing the imbibitional capacity of plant colloids.—In view of the increase in hardened plants of pentosans, especially in the hot-water-soluble fraction, and the possibility of these substances being at least partly responsible for the increased water-retaining capacity of such plants, factors which influence the water-retaining power of these and other hydrophilous colloids occurring in plants may be of great importance in relation to cold-resistance.

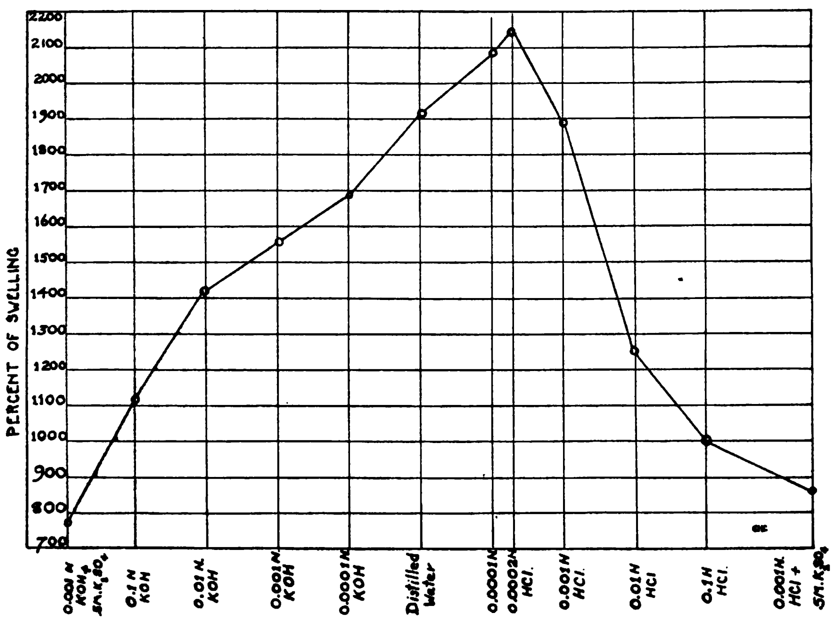

Fig. 9.—Swelling of Agar as influenced by reaction of the solution.

Acidity.—Fischer[30] showed that the power of imbibition of colloids was influenced very markedly by the reaction of the medium, as demonstrated by his experiments in which slight acidity increased the swelling of gelatin. He was able to alleviate oedema of the eye and other animal tissues by application of alkali and hypertonic sugar solutions. Fischer regards acidosis as one of the most important causes of the presence of abnormal amount of water in cells. Dachnowski[23] found that seeds of beans and corn swelled more and retained more water in N/800 acids than in water, but the amount of water absorbed and retained was not proportional to the concentration of acid, for a maximum was attained beyond which increased acidity decreases absorption. The addition of equi-molecular solutions of non-electrolytes, such as glucose and sucrose, did not increase the amount of water retained by seeds in Dachnowski's experiments. The amino-acid, glycocoll, was a striking exception in that greatly increased imbibition by seeds took place in the presence of this substance. Upson and Calvin[141] have shown that the mixture of vegetable proteins which comprises the gluten of wheat, behave in the same way as Fischer's animal proteins. They obtained maximum absorption of water in 0.01 N hydrochloric acid and 0.04 N acetic acid, with marked depression of absorption by strong acids and by salts. Mac Dougal and Spoehr[87] found a greater swelling of agar in N/100 solutions of the amino-acids glycocoll, alanin, and phenylalanin, than in water. The same workers have shown that the imbibition of proteinaceous colloids, such as gelatin, could be increased considerably by dilute acids, whereas colloids such as agar, having a pentosan base, swelled less in N/100 HCl than in distilled water.

However, brief series of tests made by the writer on the swelling of agar as influenced by the reaction, indicate that the greatest swelling of this material occurs in about N/5000 HCl. Presumably, it would require a much greater concentration of the plant acids to bring about the same degree of swelling as such a dilute HCl solution Alkalinity, excess acidity, and the presence of salts depressed the imbibitional capacity of agar very markedly. The results of a duplicate series of tests performed with shredded agar are presented graphically in figure 9.

The results obtained by Mac Dougal[82] indicate that a mixture of agar and gelatin would exhibit maximum swelling in somewhat stronger acid than would agar alone.. Since colloids of the pentosan type probably occur in plants in intimate association with proteinaceous colloids, it is reasonable to suppose that the greatest power of

imbibition would be exhibited by plant cells in the presence of slightly increased acidity. Mac Dougal and Spoehr[88] suggest that the increased acidity found in succulent plants may be a characteristic of a metabolic complex favorable to pentosan formation and to the development of succulence (a high degree of water-holding power).

In connection with the increased water-holding power of some colloids, associated with slightly increased acidity and especially some of the amino acids, it is interesting to note that Harvey[42] found a marked increase in amino-nitrogen in hardened cabbage. May it not be possible that in developing hardiness, plants form some specific amino acid which would increase the water-retaining power of the cells?

Somewhat greater titratable acidity has been found in hardened cabbage, as shown in Table 18. Determinations of the hydrogen–ion

TABLE 18.—TITRATABLE ACIDITY IN HARDENED AND TENDER PLANTS.
(cc. N/10 NaOH per one gram dry material in 100 cc. water)

Treatment	Cabbage	Lettuce	Tomato (a)	Tomato (b)
Greenhouse plants, tender	1.60	1.86	0.96	1.74
Coldframe plants, hardy	2.06	3.06	——	1.74
In coldframe, 2 weeks	1.68	——		
Grown dry in greenhouse (hardy)	1.30	——	0.96	1.00
Grown wet in greenhouse (tender)	0.82	——	0.66	1.44

concentration were also made on a few samples, but little variation could be detected by the Gillaspie method.

It seems that there is a slight increase in acidity in plants as a result of the hardening process. This change may take place only in plants possessing potential hardiness such as cabbage and lettuce since the data in Table 18 indicate no correlation between acidity and hardening treatments in the tomato. Increased acidity might also influence the water-retaining power of plant cells to such an extent as to account at least partly for the cold-resistance of hardened plants, aside from any increase in the amount of hydrophilous cell colloids. However, too few data are available to draw a definite conclusion on this point.

Salts and sugars.—It has been shown by Fischer, Dachnowski, Mac Dougal and his co-workers that the addition of salts to a solution greatly decreases the imbibitional capacity of gelatin, seeds and

agar. However, Free[29] found that gelatin swells a little more in 0.5 percent solutions of dextrose and glucose than in water, while a distinct decrease of swelling occurred in solutions of 25 percent or over. Agar was found to swell a little more in two-percent sucrose than in distilled water, whereas dextrose had little effect, except that it depressed swelling in concentrated solutions. That dilute sugar solutions do not decrease the imbibitional capacity of such hydrophilous colloids as gelatin and agar is important, since a greater sugar content is found in hardened plants. According to Goldthwaite[33] pectin and acid are prerequisites for jellification of fruit-juice, while sugar is a necessary accessory. She was able to make an excellent artificial jelly with one percent pectin, 0.5 percent tartaric acid, and three-fourths volume of cane sugar. Furthermore, in her experiments, it was shown that increasing the proportion of sugar gave an increased volume of jelly. The work of these investigators suggests the possibility of increased acidity and sugar content playing an important part in determining the state of the colloidal protoplasm.

Perhaps sudden or extreme changes in some of these factors, which influence imbibitional capacity, might exert an important influence on the water-retaining power and cold resistance of plant tissue. However, the capacity of plant organs to take up or imbibe large amounts of water must not necessarily be taken as an index of their power to retain water when exposed to conditions favoring undue water loss, such as freezing or drying.

SUMMARY.

The work of previous investigators indicates that water-loss from the cells, by the formation of ice crystals in the intercellular spaces, is most generally the limiting factor in the killing of plant tissue by cold.

Any treatment materially checking the growth of plants increases cold-resistance. In cabbage and related plants, hardiness increases in proportion as growth is checked. In tomato and other tender species, the checking treatments resulted in relatively slight increase to cold-resistance. The various means of hardening plants in these experiments have resulted in about the same type of changes within the plant.

Cabbage plants hardened by various treatments contain a larger amount of "unfree," or not easily frozen water, as measured by the dilatometer. The increment in unfree water corresponds to the

extent to which growth is checked, both of these paralleling the degree of cold resistance.

The amount of water frozen at different temperatures in leaves of varying hardiness was measured. The percentage of moisture frozen in hardened cabbage leaves at –3°C. and at –4°C. is about two-thirds of that frozen in tender cabbage leaves at the same temperature. The actual amount of water remaining unfrozen at a given temperature is greater in hardened than in tender leaves, although their total moisture content is less.

The percentage of total moisture frozen in leaves increases for each successive degree of temperature lowering, but the increase becomes rapidly smaller and smaller. The amount of water remaining unfrozen in hardened cabbage leaves is approximately a logarithmic function of the temperature.

Cabbage plants exposed to low temperatures in a coldframe for varying periods have a progressively smaller amount of water freezable at –5°C., the longer they are exposed to hardening. The percentage of freezable water decreased quite rapidly in the first four days after removal from the greenhouse, more slowly from four to fourteen days and very slowly thereafter. The rate of decrease in percentage of freezable water coincides with the observed rate of hardening. In other words, the hardening process in cabbage plants was accompanied by a proportional increase in the amount of water unfrozen at –5°C. The amount of water frozen at –5°C. is somewhat less in plants exposed to slight wilting at midday.

The effects of watering plants with M/10 salt solutions are associated with a condition of mild physiological drought. The degree of such drought is proportional to the concentration of the soil solution, which in turn is influenced by: (a) the amount of water-soluble material present and (b) the power of the soil to hold a large part of the soil moisture unfree in the pure or nearly pure state.

Hardened cabbage plants lose less moisture by transpiration per unit of leaf area than tender plants, under the same conditions. The amount of water lost by transpiration per plant for a given period is much less in hardened cabbage plants than in non-hardened plants of the same age because of: (a) the lower rate of transpiration and (b) the smaller size of hardened plants. This accounts for the fact that hardened plants can be transplanted to the field with less wilting.

The rate of water loss from hardened cabbage leaves dried in an oven at 60°C. is much less than that from leaves of tender plants. In tomato, the rate of drying is only slightly less in hardened than

in non-hardened plants. Comparing the rate of water-loss from tomato and cabbage leaves, it is found that hardened tomatoes lose water somewhat faster than tender cabbage leaves.

The lesser amount of water lost by ice formation, the lower rate of transpiration and the slower rate of water loss upon drying in hardened cabbage plants, may be explained by the hypothesis that hardening develops an increased water-retaining capacity. The water-retaining power of plant cells is due to: (a) Osmotic concentration (b) Imbibition, and may be increased by means of either or both of these factors.

Osmotic concentration of plant cells may be increased by:

(1) Decreasing the total water content.

(2) Increasing the amount of osmotically active sap solutes.

(3) Decreasing the amount of free water or conversely, by increasing the amount of unfree water held by colloidal adsorption.

Osmotic concentration as measured by the lowering of the freezing point has been found to increase on hardening plants, varying inversely with the water content. Both reducing and non-reducing sugars increase with hardening. Sugars are found to increase more in cabbage and lettuce than in tomato. The increased sugar is not sufficient to account for much difference in the freezing point depression or in the amount of water remaining unfrozen several degrees below the freezing point. The chief factor in increasing osmotic concentration in plants is considered to be the decrease in amount of free water, hence the observed increase in osmotic concentration would be a secondary result of the hardening process.

The power of imbibition possessed by plant cells may be increased by:

(1) Decreasing the total water content (or increasing the per-cent of dry matter).

(2) Increasing the amount of hydrophilous colloids in the protoplasm.

(3) Increasing the water-retaining power of such colloids by slight increase in acidity, etc.

Decreased water content accompanies a condition of greater cold resistance in plants. During the hardening process, the percentage of dry matter increases rapidly for a few days, and more slowly thereafter. The total pentosan content is greater in hardened than in tender plants, regardless of the kind of hardening treatment. The pentosan content of cabbage plants exposed to low temperatures in an open coldframe during March increases rapidly the first five

days and more slowly thereafter. The pentosan content of cabbage, kale and celery plants growing in the open garden increases as the weather becomes colder during the fall. In cabbage, kale and lettuce plants possessing potential hardiness, the fraction of the pentosan content soluble in hot water is larger than in tomato, eggplant and sweet potato, which do not possess potential hardiness. The hot water-soluble pentosan content is thought to represent more nearly the amount of pentosans in the protoplasm and these might function more specifically as water-retaining material. In the group of plants susceptible of considerable hardening to cold the increase in total pentosan content upon hardening is largely an increase in the hot water-soluble fraction, while in the tomato the hot water-soluble fraction does not increase upon subjecting the plants to hardening treatments.

CONCLUSIONS.

The experimental data show that the hardening process in plants is accompanied by a marked increase in water retaining power, and that this water retaining power is due chiefly to the imbibitional forces of the cell. The amount of water frozen in hardy plants is less than in tender plants and cells of hardy plants actually retain a larger amount of unfrozen water than those of tender plants.

It is believed that cold resistance in plants is due to the increased water-retaining power of the cells, which enables them upon freezing to retain a larger proportion of their moisture content in the unfrozen condition.

The increased water-retaining power of hardened plants is associated with the following changes: (a) decreased moisture content, (b) increased amount of hydrophilous colloids, such as pentosans, (c) increased water-retaining power of such cell colloids because of a slight increase in acidity or other internal changes, (d) increased amount of osmotically active substances as soluble sugars. The last factor probably is important only in plants hardened by prolonged exposure to cold; the first three factors mentioned may become operative in a very short time, when the activity of the plant is limited by any factor. Perhaps the same changes which increase the water-retaining power also favor greater stability of the protoplasm.

The marked parallelism between pentosan content and hardiness indicates a causal relationship. However, pentosan content alone is not to be taken as an absolute index of cold resistance, since several

factors may affect the functioning of pentosans as water-retaining substances. Salt content, acidity, hydrogen-ion concentration, sugar, moisture, protoplasmic colloids other than pentosans and perhaps, other factors constitute a varying complex which may influence water-retaining power and hardiness.

The differential reactions, when subjected to hardening treatments, of plants possessing potential hardiness as the cabbage and of plants lacking it as the tomato, indicate that the fundamental difference between hardy and tender species lies in their ability to initiate changes whereby the stability and water-retaining power of the protoplasm and consequently hardiness are increased. Hardy species and varieties of plants possess the ability to initiate such changes to a greater or less great degree, while tender species possess it to a very slight degree or not at all.

APPLICATIONS.

In view of the connection between cell water retaining power and hardiness which has been found and the correlation between soluble pentosan content and hardiness, it seems that problems dealing with cold resistance of vegetables, cereals, fruits and shrubs may be attacked from a new angle.

Furthermore, the association of water-retaining power of cells with their content of a specific material or group of materials, such as pentosans, may be important in the study of moisture relations and water movement in plants. Moreover it may lead to a better understanding of the cause and prevention of a group of physiological plant diseases usually associated with excessive water loss, such as Tipburn of potato and lettuce, and Blossom End Rot of tomato. Selection of plants for high soluble pentosan content may be helpful to the breeder of cold-resistant, drought-resistant, or disease-resistant varieties of crop-plants.

The changes of the food value of fruits and vegetables subjected to long storage may be significant, since it seems that in living plant tissues, exposed to water deficit or to cold the hexosan carbohydrates are converted into pentosans, which have a much lower coefficient of digestibility. However, the use of such vegetables as have a high water-retaining power may be important dietetically in the alleviation of certain digestive disorders.

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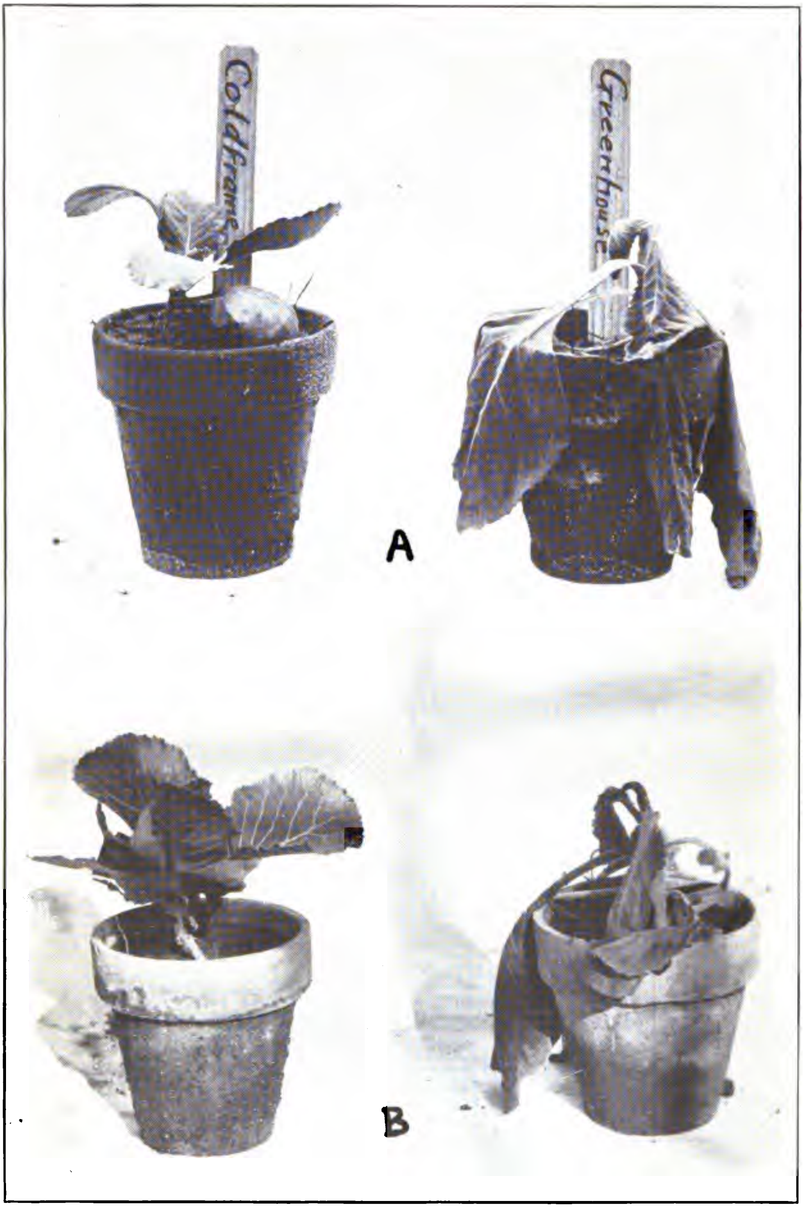

PLATE 1.—EFFECT OF EXPOSURE IN OPEN FRAMES ON COLD RESISTANCE.
A. Coldframe hardened vs. greenhouse plants frozen at –4°C. for 2½ hours. Nov. 17, 1919.

B. Cabbage plants after freezing at –8°C. for 2½ hours, March 28, 1921.
(1) Hardened in coldframe two weeks. Lower leaves broken off for samples.
(2) Non-hardened greenhouse plant.

PLATE 2.—EFFECT OF VARIATION IN SOIL MOISTURE ON COLD RESISTANCE OF CABBAGE.

A. Plants grown in greenhouse with varying supply of water; after freezing at –4°C. for 2½ hours. Nov. 17, 1919. (1) Dry grown (2) Medium dry (3) Wet grown.

B. (1) Medium-dry-grown greenhouse cabbage plants.
(2) Medium wet grown greenhouse cabbage plants after freezing at –4°C. for 30 minutes, March 28, 1921.

C. After freezing at –4°C. for 30 minutes, March 28, 1921.
(1) Watered heavily until one week before this test, thereafter wilted slightly for five days.
(2) Plant from same batch as (1) but not subjected to preliminary wilting.

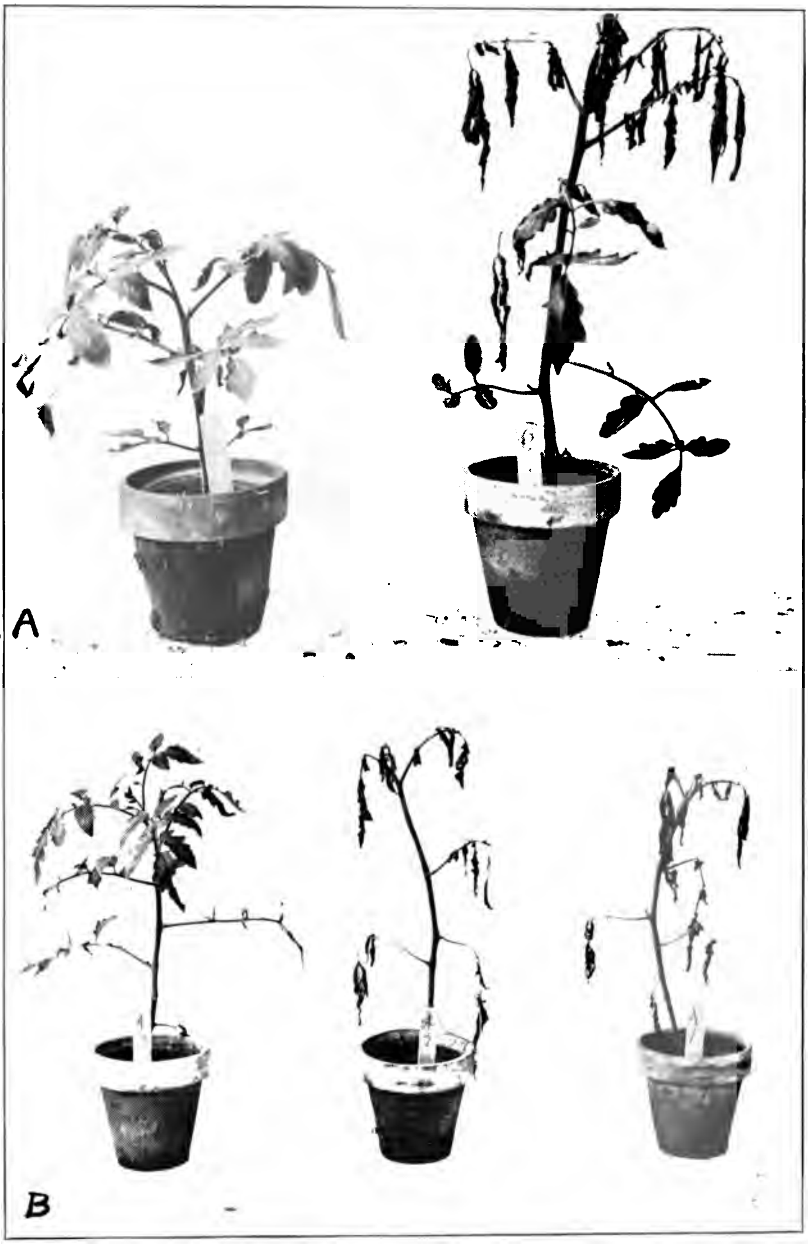

PLATE 3.—EFFECT OF VARYING SOIL MOISTURE ON HARDINESS OF TOMATO.

A. Greenhouse tomato plants after freezing at −2°C. for 2 hours, Sept. 29, 1919.

(1) Dry-grown (2) Wet-grown.

B. Greenhouse tomato plants after freezing at −2.25°C. for $2\frac{1}{4}$ hours, Sept. 22, 1921.

(1) Dry-grown (2) Medium-dry-grown (3) Wet-grown.

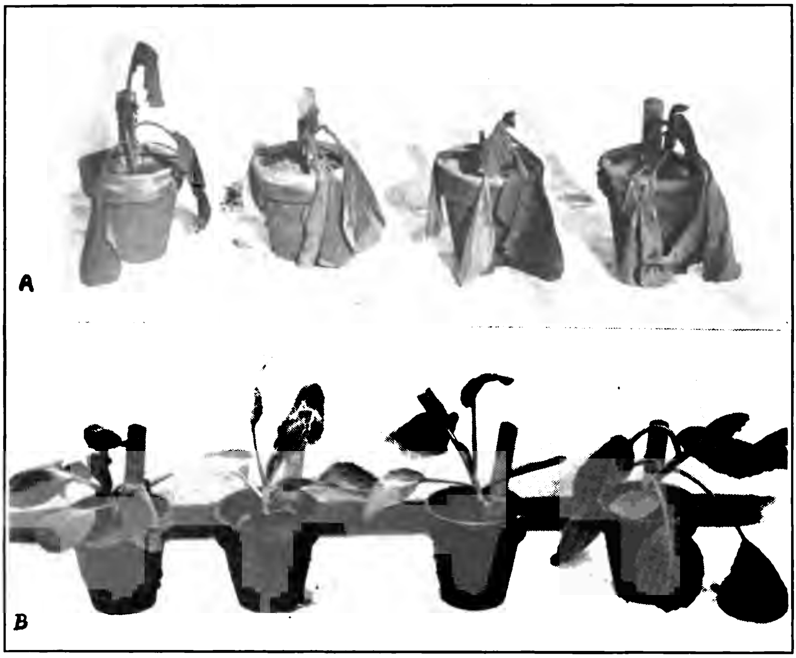

PLATE 4—.EFFECT OF WATERING PLANTS GROWN IN SAND IN GREENHOUSE WITH M/10 SALT SOLUTIONS.

A. After freezing at –6°C. for 30 minutes.
(1) NaCl (2) KCl (3) $NaNO_3$ (4) Tap water

B. After freezing at –3°C. for 30 minutes
(1) NaCl, (2) KCl. (3) $NaNO_3$ (4) Tap water.

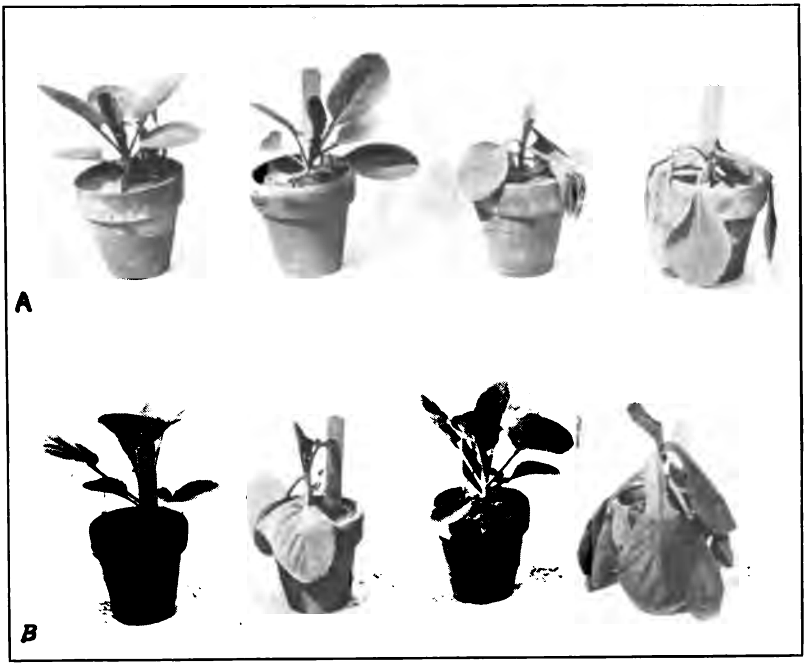

PLATE 5.—EFFECT OF WATERING CABBAGE PLANTS GROWN IN GREENHOUSE WITH M/10 SALT SOLUTIONS,

A. Grown in compost soil and watered with:
(1) NaCl (2) KCl (3) $NaNO_3$ (4) Tap water.
After freezing at –6°C. for 30 minutes.

B. Grown in Compost soil plus rotten manure, watered with:
(1) NaCl (2) KCl (3) $NaNO_3$ (4) Tap water.
After freezing at –6°C. for 30 minutes.